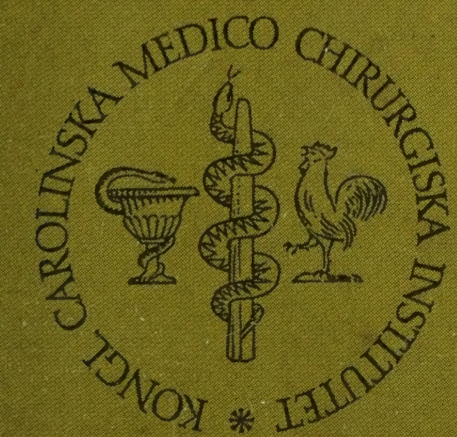


# A Reason to Be Bitter

## *Cassava Classification From the Farmers' Perspective*

Linley Chiwona-Karltun



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## **A REASON TO BE BITTER**

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Stockholm 2001

# ABSTRACT

## A REASON TO BE BITTER

### Cassava Classification from the Farmers' Perspective

*Linley Chiwona-Karltun*

Ethnographers report from both South America and Africa that cassava farmers classify cultivars as belonging to either of two groups, "bitter" or "sweet", and that farmers prefer to grow "bitter" cultivars as the staple crop. It remains contentious if the two groups and whether bitterness in taste reflects the content of cyanogenic glucosides in cassava roots. This is of importance since dietary cyanogen exposure may have adverse health effects. These studies aimed at elucidating the social and biological role of cyanogenesis in a cassava dominated farming and food system in Malawi, where small-scale farmers utilise the soaking method for processing roots from "bitter" cultivars.

In 1994 and 1995 a qualitative interview survey in 13 communities in Nkhata-Bay district, in Malawi showed that social factors were the main reasons for farmers preferring "bitter" cassava. The need to process the roots dictates rigorous planning and bitterness confers protection from theft and vermin. In 1996, determination of urinary linamarin (the cyanogenic glucoside in cassava) and thiocyanate (the main cyanide metabolite) as biomarkers of dietary cyanogen exposure, was conducted on 176 women farmers. The low urinary levels found indicated that dietary cyanogen exposure was negligible inspite of frequent consumption of food made from soaked roots from "bitter" cassava cultivars.

In 1996 two roots from 246 plants of the 10 most grown cultivars, about 24 plants from each cultivar were harvested. Farmers' scoring of bitter taste of the root tip was a good predictor of cyanogenic glucoside levels ( $r = 0.65$ ). Mean cyanogenic glucoside level in 132 roots from "cool" cultivars was 29 mg HCN eq  $\text{kg}^{-1}$  fresh weight (CI 25-33, range 1 - 123), and in 360 roots from "bitter" cultivars 153 mg (CI 143 - 163, range 22 - 661). Farmers' distinction of "bitter" and "cool" cultivars predicts glucoside levels ( $r = 0.56$ ). Taste scoring for bitterness by the taste panel was strongly correlated ( $r = 0.87$ ) with cyanogenic glucoside levels suggesting that cyanogenic glucosides convey the bitter taste in cassava. DNA fingerprinting using SSR-markers showed that farmers had a high ability to distinguish plants with specific genotypes as belonging to named cultivars. Their distinction of "bitter" and "cool" cultivars with high and low levels of cyanogenic glucosides appears to have influenced the genetic pool of cassava in this area since the genotypes of the two groups separated into two clusters in principle component analysis.

Single women's food security was particularly compromised if they did not opt to grow the "bitter" cassava cultivars. Processing bitter roots was not a problem *per se*, rather the need for mechanised mills was perceived as the rate-limiting factor. Farmers' ethnoclassification and experience enables them to have more benefits than disadvantages from cyanogenesis. These findings have implications for cassava breeding and extension programmes.

*Key words:* Cassava, bitter, sweet, cyanogenic glucosides, cyanide, sensory analysis, taste, theft, women, food security, SSR-markers, molecular genetics, plant morphology, Malawi, Africa

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*Dedicated to*  
*my father Peter Harry Chiwona*  
*&*  
*my mother Makwegho Irene Musopole*

*“Tell me”, he wrote, “tell me what you eat and I will tell what you are”*

*Jean-Anthelme Brillat-Savarin (1755-1826)*  
*La Physiologie du Goût (The Physiology of Taste)*

*For Karin and Nora*

# PAPERS IN THE THESIS

The present thesis is based on the papers listed below. They will be referred to by their roman numerals in the text. The papers have been printed in the thesis with permission from the publishers.

- I. Chiwona-Karltun L, Mkumbira J, Saka J, Bovin M, Mahungu NM, Rosling H.  
**The importance of being bitter - a qualitative study on cassava cultivar preference in Malawi.**  
*Ecology of Food and Nutrition* 1998: **37**; 219-45.
- II. Chiwona-Karltun L, Tylleskär T, Mkumbira J, Gebre-Medhin M, Rosling H.  
**Low dietary cyanogen exposure from frequent consumption of potentially toxic cassava in Malawi.**  
*International Journal of Food Science and Nutrition* 2000: **51**; 33-43.
- III. Chiwona-Karltun L, Brimer L, Saka J, Mhone A, Mkumbira J, Johansson L, Bokanga M, Mahungu NM, Rosling H.  
**Bitter taste of cassava roots strongly correlates with cyanogenic glucoside levels.**  
*Journal of the Science of Food and Agriculture*, in press 2001
- IV. Mkumbira J, Chiwona-Karltun L, Lagercrantz U, Mahungu NM, Saka J, Mhone A, Bokanga M, Brimer L, Gullberg U, Rosling H.  
**Classification of cassava into “bitter” and “cool” in Malawi: from farmers’ perception to DNA characterisation.**  
Submitted for publication 2001

## Photograph descriptions

*Front cover:* The oldest respondent interviewed (from Usisya Village) in the midst of preparing *kadonosya* from soaked and pounded roots of “bitter” cassava cultivars. The decorative balls are designed with finger imprints and left to sun-dry for a week on a rock (Study I).

*Back/top:* The author expectantly waiting for the farmer to *kucheta* (score the degree of bitterness of the tip of a cassava root) during the field survey (Study III & IV).

*Back/middle:* A *tawali* (soaking pond) in continuous use since 1963 for fermentation of cassava roots of “bitter” cultivars (Study I).

*Back/bottom:* Chief Fumukazi Mtepelera, the local community leader of Mgodì explaining to the research assistant how she is able to tell if the poison has disappeared from the “bitter” cassava roots during fermentation (Study II).

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## PRELUDE

*“Umututumushi, gho mayabo tukusubalila kuno ku Kapoka. Nandi atili atwe apo na apo utanginula wimwene nashiku, lole amashiku agha amyabo ghata kwina inghani ngati bwira”*. In English: *“Umututumushi, now that is the cultivar that the village of Kapoka truly relies on. When it produces the roots can extend “from here to there” and you cannot harvest it by yourself, but nowadays cassava in general does not yield like it used to”*.

My late grandfather Lyson Kafyanya Mbisa told me these words. He was my first key-informant in 1993. When he died at an old age he left behind the legacy of cassava, its past and present importance in the lives of the a Marawi people. I video recorded my interview with him and seven years later I am attempting to narrate the reasons why not only cassava but “bitter” cassava is crucial in alleviating hunger in his village in Chitipa district, but also in other parts of the world. This first interview captivated my interest in the crop and I sought to find out more and in my second interview with Stanley Ambokile Muspole in Iyela I heard: *“umbundumale ali nuwufu wingi naloli sona akwikutisha leka ukungwera namishi”*. In English: *“Umbundumale (you cannot finish it) has a lot of floury dry matter and it is extremely filling. One thirsts for water after eating it”*. Why I thought to myself, they talk about the cassava cultivars using names that actually describes their characteristics, what else can I learn from them?

During my studies at Uppsala University I had heard many a seminar on cassava presented by my colleagues. What is it that is so perplexing about cassava I wondered? I had grown up with it, eaten and survived and had scores of relatives, not to mention the other 500 million around the world, that are still growing and talking so highly of it. These cassava growers seem to be at peace with this crop so what was all the hype about cyanogenesis. You see, mankind has devised ways of “taming” the poison to a point that the “bitter” and toxic cassava cultivars are preferred. Is there a certain lure with the poison in cassava that provides those that grow cassava some hidden advantage. This was my point of departure for this thesis *“in quest of bitter cassava – whither the reasons”*.



# INTRODUCTION

Cassava (*Manihot esculenta* Crantz, *Euphorbiaceae*) is the prime source of dietary energy for well over 500 million people dwelling in the tropics(1-3). Cassava's primary role in agriculture is the provision of starchy tuberous roots(1, 4-7). To a lesser extent cassava is grown for its leaves(8-10), that can be boiled and used as a vegetable. Outside the tropics trade of cassava is limited to animal feed, starch and minor amounts of tapioca flour (an Amazonian Indian *Tupi* word meaning "absence of juice"). The latter is used as an ingredient in puddings and sauces in countries outside the areas of cassava production(11).

The cassava plant is a perennial shrub that attains about 2 meters in height at maturity. Its cultivation is confined to the tropics between the latitudes 30° north and 30° south. Cassava is propagated by 2 - 4 decimetre long stem cuttings(12-16). During the first four months after planting the above ground parts of the plant develop and thereafter starch accumulation occurs in the roots. In the dry season, the roots stop growing and the starch content increases due to reduction in moisture content. This makes the dry season the best period to harvest the roots(16). Cassava roots are 50 – 70 cm long, usually have white or yellow coloured flesh and a thick brown peel. The plant develops an average of three to six roots per plant and each root may weigh 2 – 5 kg(12). The absence of fixed planting or harvesting period makes cassava attractive as a plant for the tropics. Cassava produces well in a variety of soils except in waterlogged conditions. Cassava is an efficient extractor of soil nutrients even in worn-out soils(16) and it is very drought tolerant although lack of water compromises the yields.

Ironically, the very efficiency of cassava as a crop has been perceived negatively by those thinking that cassava contributes to "laziness" of traditional rural societies(13). A cassava field needs weeding only once and harvesting can be achieved by simply pulling the plant from the ground if the soil is sandy. The amount of labour required is about 20 days per metric tonne much less than that of other cereal crops(15, 17). The subsistence farmer may harvest anywhere between 2.5 to 30 tonnes per hectare(15) and the world average is estimated at 10 tonnes fresh weight per hectare(18). The potential of cassava has been shown by research at the International Institute of Tropical Agriculture (IITA) in Nigeria. The crossing of a cultivated cassava variety and a wild rubber species introduced to Africa resulted in a variety that yielded 70 tonnes per hectare compared to the 6 tonnes that cassava farmers in Africa usually harvest(19). Cassava has few diseases and pests but when they attack they can be ferocious. The main problems in Africa are mosaic virus, mealy bug, bacterial blight, brown streak virus and varying types of root rot(16, 20). New strains of the cassava mosaic virus have of late been a major cause of distress for cassava farmers in East Africa, especially in Uganda(21).

The word cassava seems to have been derived from the word *casabe*, an Amazonian Indian *Taino* (*Arawak*) word for cassava bread(6, 22). Cassava is the name used in the West Indies and most of the English speaking world(6, 11, 22). In American English it is also known as manioc. According to Sauer(12) the word *manioc* comes from the *Tupi-Guarani* language and it is also the French word for the plant. In Brazil, cassava in Portuguese is known as *mandioca*(5, 13) and in Spanish speaking Latin America it is known as *yuca*. In the Pacific Oceanic it is recognised as *tapioca*(2).

## ORIGIN AND BOTANICAL TAXONOMY

It has proven difficult to establish the origin of cassava using traditional archaeological evidence. The reason is that cassava growers did not store the seed of the plant. The major planting materials were the non-storable stem cuttings. Only perishable root products were stored. Therefore, since no persistent parts of the plant can be identified in graves or other archaeological remains, archaeologists have used remains of starch particles left behind on the processing and cooking equipment. These have been identified to be of cassava origin and have aided in postulating the most likely origin of

cassava(23-25). Recent studies in Brazil using molecular genetics, have shown that Brazilian *Manihot esculenta* subsp. *flabelliofolia* species from the Amazon Basin are the most likely source and sites of domestication(26-28). Based on starch grain particle analysis left in the crevices of some of the archaeological equipment used for processing cassava, it is believed to have been domesticated in Amazonia about 8000 years ago(29) and it seems that cassava domestication thus constitutes one of the independent origins of human agriculture(29, 30).

Cassava is one of about 100 species of the genus *Manihot* that also includes several rubber-producing plants(31). This genus is a member of the *Euphorbiaceae* family(24). Of all plants in the *Euphorbiaceae* it is only *Manihot esculenta* that produces the tuberous roots that have lead to its domestication(5, 32, 33). Cassava is now recognised as constituting the species *Manihot esculenta* Crantz(27) but this has not always been the case. Crantz established the name *M. esculenta* in 1766 based on the description of Linnaeus who in 1753 placed cassava as the only species in the genus *Jatropha* with the name *J. Manihot*(34).

In 1827 Pohl conducted extensive original field surveys on the genus *Manihot* in South America. In his taxonomy he distinguished between the poisonous cassava designated as *M. utilissima* and the non-poisonous cassava that he named *M. aipi*(34, 35). Studies in Jamaica a century ago, showed significant differences in the yield of hydrocyanic acid from different parts of roots of “bitter” and “sweet” cassava cultivars and this distinguished the two types of cultivars in the taxonomy(36, 37). In 1919 Zehntner divided the Brazilian cultivars into two groups based on high and low yield of hydrogen cyanide from the roots(34). However, in 1938 the botanist Ciferri concluded that there was no sufficient reason to regard the “bitter” and “sweet” cassava as separate species. He based this on his studies of 19 varieties in the Dominican Republic(38). In 1965 Rogers tested 100 Jamaican cultivars and found the cyanogenic glucoside levels to constitute a continuum(39). He thereafter suggested that all cassava should be classified under the name *Manihot esculenta* Crantz.

**Box 1. Botanical Terminology**

<b>Species</b>	<b>Taxonomic group:</b> a subdivision of a genus considered as a basic biological classification and containing individuals that resemble one another and that may interbreed
<b>Genus</b>	<b>A set of closely related species:</b> a category in the taxonomic classification of related organisms, comprising one or more species. Similar genera are grouped into families.
<b>Variety</b>	<b>A subdivision of species:</b> a rank used in classifying living things, especially plants, that is subordinate to species but superior to form. Varieties of a species generally have certain distinguishing characteristics, such as a particular flower colour, and may arise naturally or through deliberate plant breeding.
<b>Cultivar</b>	<b>A plant variety</b> that has been incorporated and is grown within a farming system(14)
<b>Genotype</b>	<b>Genetic makeup:</b> the specific genetic makeup of an organism, as opposed to its physical characteristics (known as phenotype)

Recent studies confirm that all cassava cultivars belong to the same species and that there is a high likelihood that this species has hybridised with other *Manihot* species(28). Like with many other crops small-scale farming communities growing cassava give local names for the numerous cultivars used within their farming system.

Whenever cassava cultivators migrated from one area to another they took their cultivars with them. The Caribs and Arawak Indians moved from South America to the Caribbean(37, 39, 40). One cultivar could move back and forth over large distances. A cultivar by the name of Brazil, was taken from Brazil by the Portuguese to Indonesia and brought back to Cuba and thereafter taken up as an ideal producer in Costa Rica(39).

Cassava plants may be found growing wild in “abandoned fields” or “road-side”. Studies in Guyana(41) show that although cassava is strictly vegetatively propagated as an agricultural crop,

Amerindian farmers recognise “volunteer” seedlings that arise from a bank of viable seeds that may have been stored for long periods in anthills and finally dispersed by ants. If such “wild” sexually reproduced seedlings have qualities that are deemed desirable by the farmers they are incorporated as a new cultivar into the farming system. This may be an important function in the evolution of cassava as a domesticated plant(41, 42).

It seems as if in many, if not most of the cassava growing areas, the cultivars are classified into two groups that in the English literature have been referred to as “bitter” and “sweet”, respectively(12, 24, 43). There is consensus in the literature that roots of “bitter” cassava cultivars tend to contain higher levels of cyanogenic glucosides than the roots of “sweet” cultivars(44-50).

## **CASSAVA IN THE FARMING SYSTEM**

Cassava farming systems may be divided into pioneer, permanent and shifting cultivation or swidden agriculture systems(16). The pioneer system refers to the new introduction of cassava in an area. Cassava cultivation first developed in South-East Asia by inter-cropping in young rubber-tree plantations(16, 46). A permanent system implies practising soil conservation and soil fertility management, as well as crop rotation and measures to prevent soil erosion. This system is mostly synonymous with a high degree of mechanisation and high economic input and is rather common in South America(51).

The main cassava farming system in Africa is known as shifting cultivation or slash and burn system(52). Shifting cultivation requires that enough land is available so that after a few years of growing on one piece of land it can be abandoned and left fallow for several years while cultivation continues after burning or clearing another piece of land(52). The cassava shifting cultivation farming system of Africa is presently undergoing a dramatic change. Population growth is lending towards land scarcity and the fallow periods must be shortened or completely abandoned(19, 52, 53). The result is a “collapse” of the shifting cultivation. The soils will become depleted and yields fall(16). Cassava cultivars that had good yields in the true shifting cultivation have reduced their yields from 10 tonnes/ha to 6t/ha or even lower(54).

Due to population growth sub-Saharan Africa will continue to experience a rapidly increasing demand for food and feed(55). This will account for increased demand for cassava because of its higher yielding capacity. Economic decline and rural to urban migration in search of livelihoods are all factors that can play a major role in increasing the demand for cassava(56).

Areas with cassava dominated shifting cultivation systems have often experienced a shift from cereals to cassava due to food insecurity and hunger(55, 57). This is especially true in parts of Eastern and Southern Africa with low rainfall. The advantage of changing from a cereal based cropping system to cassava is due to its drought tolerance(19). The fact that cassava can be left in the fields for a long time also makes it invaluable to communities with seasonal migratory patterns for employment purposes or displacement due to war(40).

There is a strong association between increased production and consumption of cassava and increased incidence of HIV/AIDS. This process is part of the feminisation of agriculture in Africa(58). Turning to cassava that has less labour and economic pre-requisites attached to it is a coping strategy that has historically been documented. In Niger following the influenza pandemic between 1918-19 yams ceased to be the main staple food and were replaced by cassava(59). This was due to the lack of men needed to perform the more physical chores of felling down trees and clearing the agricultural fields required for yam production.

Today, in countries like Tanzania and Malawi, the main role of cassava is to bridge the gap between the “lean” season and that of “plenty”(60, 61). This phenomenon is not peculiar to Africa alone, it has also been reported from Asia and Latin America(2, 19, 55).

# CASSAVA IN THE DIET

Cassava is the second most important staple food in Sub-Saharan Africa providing an average of 285 calories per person per day(18). Cassava roots are mainly eaten after having been processed into flour, mash or granules that are used to prepare a dumpling like porridge. This staple food is eaten together with a relish made of vegetables and other plant or animal products. In their simplest form cassava roots from the “sweet” type of cassava are either eaten raw, boiled, roasted or cooked in a stew as a pot vegetable. On the contrary, the roots from the “bitter” cassava cultivars are without exception subjected to elaborate processing that entails peeling, grating, soaking, fermenting or drying of the roots. Processing is conducted to achieve the desired organoleptic qualities of the staple food while simultaneously reducing the levels of cyanogen compounds to safe levels. Processing requires planning since it takes five to ten days before the product is proclaimed safe and ready to eat(62). The products made from cassava roots are described in the section on processing in this thesis.

Cassava roots on fresh weight basis contain only 1-2% protein and 0.3% fat(15). Cassava roots proportionally are lower in protein(47) compared to all the other major staple foods (table 1). Cassava roots contain some thiamine, riboflavin, niacin and ascorbic acid at levels similar to those found in rice. Due to the low protein content cassava has been viewed as a cause of malnutrition and kwashiorkor, also called red babies(7, 63, 64). Cassava is the main staple food in many areas where livelihoods are inextricably woven with rivers, or close to lakeshores and coasts(7, 40, 65, 66). The fishing in these areas have provided the much-required protein in the cassava based diet. It would thus seem that cassava’s role is first and foremost to provide calories and the essential micro-nutrients are supplemented by game, fishing and consumption of other protein rich plants(22).

**Table 1. Calories and protein provided from major staple crops utilised for direct human consumption in Sub-Saharan Africa\***

Crop	Calories/person/day	Protein/person/day (g)
Maize	337	8.6
Cassava	286	2.0
Sorghum	202	6.0
Rice milled	175	3.6
Millet	137	3.3
Wheat	121	3.6
Yams	78	1.2
Plantains	61	0.5
Sweet potato	33	0.4
Potatoes	10	0.2

\* FAO statistical database accessed 9 August, 2001(18)

## Cassava leaves

Cassava leaf consumption is part of many cassava farming and food systems(8, 67-69). In a study across Africa 81% of included cassava growing communities reported consuming cassava leaves(53). It is very common in Africa to cook cassava leaves with some fish, meat, groundnuts or seeds of some sort. This combination takes care of the missing amino-acids(70). Essentially this means that cassava is not a poor source of protein if the leaves are also consumed(10). However, there is limited research done on the bio-availability of vitamins and minerals in the leaves.

Besides protein the leaves contain vitamins such as vitamin C, beta-carotene, vitamins B1 and B2 niacin and minerals including ferric oxide and calcium(47, 67, 71). Many farmers that grow and consume cassava consider it to be a complete crop, since the roots provides the bulky energy and the leaves provide the stew that goes with it. Cassava leaves are reported on a fresh weight basis to contain 8 – 9% of crude protein, and 4 % of essential amino acids but very little sulphur-containing amino-acids(47, 72, 73). The leaves lack methionine and have some traces of tryptophane but contain excellent levels of lysine, a limiting essential amino acid in cereal based diets(72). Another study reported cassava leaves to have an amino-acid profile that was sufficient in all the amino-acids(74). A recent study from Ghana showed that feeding pre-school children with cassava leaves and added fat once a day for three months improved the serum retinol levels. This is of importance in preventing Vitamin A deficiency in the tropics(68).

## CYANOGENIC GLUCOSIDES IN CASSAVA

Several plants used as food contain natural toxins. Examples include beans, peas, sorghum, potatoes and cassava. Our ancestors that domesticated these plants solved this problem in either of two ways. The first way was to select for varieties with negligible toxin levels. The second way was to devise processing methods that reduced toxins to negligible levels(4). Cassava is a major example of the latter strategy, i.e. removal of potential toxins by processing.

The main cyanogenic glucosides in cassava are linamarin(45, 75) and smaller amounts of lotaustralin(76). They occur in the proportions of about 10:1. Linamarin and lotaustralin are synthesised from the amino-acids valine and isoleucine. These cyanogenic glucosides(77, 78) can be found throughout the whole cassava plant with the highest concentrations in the young leaf shoots(79), followed by the petioles, stems and roots(80). Synthesis of the glucosides mainly occurs in the leaves and they are transported to the roots although there is little synthesis in the root as well(81). Within the root, the peel contains more cyanogenic glucosides than the edible flesh(49, 82). Genetic and environmental factors concurrently determine the glucoside levels in the roots(83). Nowhere in the world has there been identified a cassava cultivar without any cyanogenic glucosides, except as an anecdotal statement. This statement refers to the existence of cyanide-free tubers in Indonesia before World War II but this cultivar was subsequently lost, page103(84).

All cyanogenic glucoside-containing plants also contain enzymes for the decomposition of the glucosides. In cassava this endogenous enzyme is called linamarase(85, 86). The glucosides and the enzymes are stored in separate compartments within the plant cell, the former being stored in the cell wall and the latter in the cell vacuoles(87). In order for cassava tissues to liberate hydrogen cyanide (HCN) contact has to be made between the substrate linamarin and the enzyme linamarase. This is done by bruising the tissues or by any other process that ruptures the cell structure such as grinding, grating, soaking and fermentation, freezing, drying or the addition of chemical agents(88, 89). The hydrolysis of the glucosides yields glucose and cyanohydrins that remain stable at a pH <5 and at low temperatures. An increase in temperature or pH results in spontaneous breakdown of the cyanohydrins to acetone and HCN(89). The breakdown may also be facilitated by the enzyme hydroxynitrile lyase that is expressed in the leaves but not in the roots(90). This process of releasing HCN is referred to as cyanogenesis(91). The glucosides, cyanohydrins and HCN are collectively referred to as cyanogens because they are all capable of forming the cyanide ion  $CN^-$  that is the toxin.

Factors that may affect the level of cyanogenic glucosides range from the age of the plant(88, 92, 93), growing conditions(94) to genetic factors(15, 34, 48, 83, 95)]. According to Bokanga(49) three roots per plant and four plants in each replication are required to attain a good estimate of the cyanogenic potential.

## THE SAGA OF BITTER AND SWEET CASSAVA

Already in 1954 Bolhuis found it difficult to compare reports about “bitter” and “sweet” cassava because most authors had failed to define what they meant by “sweet” and “bitter”(92). This comment remains valid as many authors have not explicitly stated if they were referring to folk taxonomy, botanical, sensory, chemical or genetic observations when labelling cassava cultivars as “bitter” or “sweet”.

Ethnographic studies consistently report that the indigenous Indians in Amazonia classify cassava cultivars as belonging to two major groups(4, 5, 14, 22, 43, 50, 96-98). Of particular note are the studies with the Tukanoan Indians that have shown that they systematically classify cassava cultivars as *kii* or *makasera*(98). Cultivars of the group termed *kii* are regarded to yield “bitter” roots that require elaborate processing prior to consumption while cultivars of the *makasera* group are perceived to be “sweet” and can simply be eaten boiled or baked. The two types of cassava cultivars are used as staple food (“bitter”) and pot vegetable (“sweet”), respectively. The use of “bitter” cultivars as the staple food requires knowledge of processing techniques and equipment as well as craftsmen to produce the equipment for processing. Archaeological findings suggest that this knowledge has been the decisive factor for the spread of the “bitter” cultivars. The “sweet” cassava cultivars have spread more expansively(7, 24, 25). Cassava cultivars continue to be classified into two groups referred to as “bitter” and “sweet” by many of the farming communities in South America(96). How can it be that this folk taxonomy continues to exist despite scientists for many decades perceiving the crop as one single species, *Manihot esculenta* Crantz, with continuous variation of cyanogenic glucoside concentrations in different genetic varieties(34, 39, 97, 99). All the more perplexing since the distinction between “bitter” and “sweet” cassava cultivars has no taxonomic basis when plants are examined morphologically by botanists(23, 34).

In the early thirties several studies(46, 92, 100) concurred that “bitter” tasting roots are invariably poisonous, whereas “sweet” tasting roots may be either harmless or poisonous. There was an agreement that bitter taste was more pronounced in raw cassava roots than when they are cooked and a taste for bitterness should therefore always be done on raw roots(79, 92, 100). Based on the lethal dose of hydrogen cyanide being around 50-100 mg for an adult(47) safe levels of HCN yield from cassava roots were defined knowing that poisoning from cassava consumption is rare in societies where the traditional processing methods are adhered to(98, 101).

### *The geographical distribution of bitter and sweet*

There is documentation of different geographical distribution of “sweet” and “bitter” cassava cultivars(53, 103, 104). It is noted that the “sweet” cultivars have a wider range of distribution than the “bitter” ones, i.e. there are many areas where only “sweet” cultivars are grown and eaten(6, 14) (<http://www.globalcassavastrategy.net>). It is suggested that the “bitter” cassava was used as provisions for the warfaring tribes in South America. Since it required knowledge of processing it was possible for those communities that had that knowledge to grow such dangerous crops knowing that their potential enemies did not have this knowledge(43).

Observations in both South America(4, 12, 14, 15, 22, 43, 105) and Africa(6, 52, 53, 106) have shown that where cassava is of major importance as a staple food the “bitter” cassava comprises a higher proportion of the farming system and the “sweet” cultivars play a minor role. Where “sweet” cassava cultivars are grown in higher proportions or if they constitute the only cultivars than the “bitter” cultivars they are almost always a part of a more diverse farming system. In parts of South America plants of “sweet cultivars” are often inter-cropped with maize or other crops and are grown in small patches in the middle of a field with other crops. When both “bitter” and “sweet” cassava cultivars are grown, the “bitter” cassava usually is planted on the outer fringes whereas the “sweet” cassava is grown in the middle or interspersed between the “bitter” cassava. The “sweet” cultivars are more often than not grown near the homestead.

The Portuguese brought cassava to Africa from South America around 1558(6). Slave traders planted cassava on the coast and it is reported that some slaves died of cassava poisoning (107, 108). During the colonial era the British colonialists made it a law to grow cassava as a famine reserve-crop(40, 109). Cassava gained its importance in Africa as an anti- famine food and as a coping strategy against lean periods(19, 37, 102, 106, 110). However, the historical diffusion of cassava, especially the diffusion pattern of “bitter” and “sweet” cultivars in Africa continues to evade us due to lack of research.

**Table 2. Ethno and scientific classification of cassava cultivars into two main groups the “bitter” and the “sweet”**

Bitter	Sweet	Area	Reference
Bitter	Sweet	South America; Africa	Nordenskiold, Jones, Purseglove, Nweke(4, 6, 14, 53)
High cyanogenic potential	Low cyanogenic potential	South-East Asia; Oceanic	Koch; Greenstreet(46, 100)
Poisonous	Innocuous	Oceanic	Bolhuis(92)
<i>Manihot utilissima</i>	Boniato	South America	Sauer(12)
Red	White	South Africa	Velcich(102)
<i>Manihot utilissima</i>	<i>Manihot dulcis</i>	South America	Rogers(39)
<i>Manihot utilissima</i>	<i>Manihot palmata</i>	South America	Rogers(39)
<i>Manihot utilissima</i>	<i>Manihot aipi</i>	South America	Rogers, Renvoize(39, 103)
Mandioca	Macaxeira	South America	Lathrap, Rogers(34, 43)]
Amarga	Dulce	South America	Rogers(34)
Mandioca brava	Aypim	Brazil	Moran(13)
Kii	Makasera	South America	Dufour(50)
Poisonous	Non-poisonous	South America	Dufour(50)
Brava	Dulce	South America	Dufour(50)
Mandioca	Aypi	South America	Dufour(98)
Bitter	Baridi (cold)	Tanzania	Kapinga(60)

### A preference for bitter cultivars

In 1986 Fresco wrote on page 153: “throughout Africa, bitter varieties are much more common than sweet ones, notwithstanding the fact that sweet varieties have been promoted by some colonial administrations out of concern with the toxicity of bitter varieties. There is no satisfactory explanation in the literature for the predominance of bitter varieties”(52). Indeed, the preference for “bitter” cassava seems to be an exception to the general rule that human beings select plants that are less poisonous(111). Several reasons have been suggested as to why cassava is the only toxic staple food and why in spite of this it remains the fourth most important staple crop(19). Interestingly enough there is preponderance by humans to grow cassava that has high cyanogenic glucoside content in spite of sweet cultivars with low cyanogenic glucoside levels being available (14, 50, 96, 99).

One possible reason is that “bitter” cassava cultivars have higher yields than “sweet” cultivars let alone other crops(96, 99, 112). This has been reported as anecdotal statements that farmers have been quite emphatic about higher yields in “bitter” compared to “sweet” cultivars, despite research at agricultural stations not being able to show an association between yield and cyanogenic glucoside level(113).

A second reason is that end-products from bitter cassava cultivars may have preferred qualities. These could be factors such as higher starch content or other appearances of the final product (11-14, 22, 43, 50, 52, 62, 97, 114).

The third reason pertains to the storage qualities(115) of processed products. The processed products made from “bitter” cultivars are stated to have better storing qualities(22, 52) especially regarding resistance to weevil infestation(116). In areas where harvesting is done all at once the importance of good storage qualities plays a big role and in those areas “bitter” cassava seems to be preferred.

A fourth reason for preference of “bitter” cultivars is their ability to keep away predators(14, 99, 117). Since one of the advantages is to leave cassava in the ground for up to three years, it is thus desirable to have a toxic crop than a “sweet” one since baboons, monkey, agoutis and peccaries would end up eating the crop(99, 118).

The fifth reason is protection from thievery. This relates to involuntary social changes in society that has lead to escalating theft of food crops. This has been reported to result in preference for “bitter” cassava(60). This practice of growing “bitter” cassava to prevent theft of cassava has been observed very far back in time(46, 100, 104). Many farmers do not feel food secure in growing only “sweet” cassava cultivars(119) since when left in the ground for long periods the risk of being stolen is very high(65, 118, 120).

The sixth reason, often cited in literature, is the possible protective effect of the toxin against insects(99, 121, 122). A study comparing the attacks of insects on maize and cassava showed that maize was more susceptible than cassava and that the “bitter” fared better than the “sweet”(116). Similar observational studies in Uganda show that indigenous crops such as millet and sorghum fail to survive locust attacks but cassava copes much better, possibly due to its inherent chemical defence(40). There are on-going studies looking at the possibility of cassava without cyanogenic glucosides(85, 90) despite the little understanding of why cassava still remains the only toxic staple food. The possible chemical plant defence can have major economic impact for farmers(111, 123, 124). The role of cyanogenesis as a possible chemical defence in cassava is perceived to be a central theme(99).

Studies on the farmers’ preferences for potentially toxic plants have rarely been carried out in Africa(60, 125) and when conducted the farmers’ preferences are not analysed with a full understanding of their social and cultural environment(118). Farmers’ preferences for cultivars with specific characteristics have been found to have profound implications for breeding and agricultural extension services(126).

*Taste in relation to cyanogenic glucosides*

Koch(100) classified cassava roots according to their degree of toxicity based on Boorsmans statement before 1905(92) that 50-60 mg HCN is the theoretical lethal dose for an adult man weighing 50 kg(100). This led to the publication by Bolhuis of Koch’s arbitrary classification based on the yield of hydrocyanic acid from peeled raw roots(92) (table 3).

**Table 3. Kochs’ 1933 arbitrary classification of cassava toxicity based on Boorsman’s statement in 1905 as reported by Bolhuis(92)**

Cyanogenic potential	Risk assessment
Less than 50 mg HCN/kg fresh root	Harmless
50 – 100 mg HCN/kg fresh root	Moderately poisonous to poisonous
More than 100 mg HCN/kg fresh root	Very poisonous

It is well established that cassava roots contain varying amounts of cyanogenic glucosides and that there is a wide range of bitterness of the roots. Although it is agreed that the toxicological potential of cassava is mainly due to the occurrence of cyanogenic glucosides(80), there remains discord as to whether it is the glucosides *per se* that convey the bitter taste(49, 106, 128). In a small study of six cultivars King and Bradbury(129) recently found that linamarin is the main contributor of bitterness in the parenchyma of cassava roots. They also identified another bitter compound contributing to the bitterness of the peel of the cassava root but not to the bitterness of the parenchyma, i.e. the edible parts. This is compatible with how the farmers that grow and consume cassava associate the bitterness of the fleshy part of the raw or boiled cassava roots. Similar findings have been published by researchers over one hundred years ago(44, 130, 131). In these studies the authors stated that a retention

of high glucosides after cooking meant that the roots were not safe and that they retained a bitter taste(79) as a risk indicator.

Since taste and glucoside levels vary between roots of the same plant it is imperative that each root be tasted for bitterness and assessed for its HCN potential to study the correlation between taste and toxicity. It appears that the main problem in many published studies lies in the definition of how to test the relationship between taste and toxicity. Several of the studies arguing for and against causal relationship between bitter taste and cyanogenic glucoside have used methodologies that make comparison of results difficult. There are some studies that have found no correlation between taste and toxicity(128, 131, 132). What is most interesting in these studies is the way the cassava samples were defined, collected and analysed. The three studies finding no correlation appear to have used different roots for measuring cyanogenic potential and taste of a cultivar instead of comparing between taste and toxicity in the same roots.

There are other studies that have shown a positive association between bitterness and cyanogenic glucoside levels(93, 133-135). Furthermore, many cassava farmers equate bitter taste with toxicity and danger to determine whether roots need to be processed before consumption(97, 125). Most noteworthy are the studies done with the indigenous communities in South America(50), showing that farmers correctly associate the poisonous cultivars called *kii* with the quality of being bitter in taste.

## CASSAVA PROCESSING

Ethnographers have reported that not only is processing conducted for detoxification but that it is mainly done to attain the preferred food products, particularly cassava flour(96). In addition processing improves shelf life, reduces bulk during transportation and improves palatability of the prepared dishes(4, 104, 136, 137). It appears as if detoxification is mostly achieved “automatically” as the roots are in the process of being processed for other purposes. Without exception processing is almost exclusively done by women be it in Amazonia, Asia or Africa. Only when it is highly mechanised does one see the involvement of men in the processing activity(62, 114, 138, 139).

### *Utilisation techniques by native Amazonians*

Detailed descriptions of cassava processing come from the anthropological studies in the Amazonia(4, 5, 12, 43, 105, 137, 140, 141). The processing techniques entail a sequence of peeling, grating, washing, fermenting, drying, frying or baking(4, 137, 142, 143). Thereafter, the pulp is made into cakes (*casabe*) or processed into flatbread or into a granular meal (*farihna de manioc*).

Traditionally processing of “bitter” cassava into granular meal is done by using sophisticated carved wooden graters or thorns on the catizal palm imbedded in palm wood plants(144). Thereafter the mash is pressed in long twilled basket presses called the *tipiti*(43). In some areas in the lower Amazonas, cassava farmers have used quartz flints or diamond chips in their graters and it is said that the discovery of diamonds in cassava graters is what begot the diamond industry in Brazil(140). However, this traditional processing equipment is being replaced with more modern tools especially in areas where cassava has been incorporated into the modern sector. Cassava roots are also processed into starch(15, 137, 142). The water that is used for washing and rinsing the starch is boiled and made into a drink that is called *mingao*, a very popular drink among the indigenous Indian groups(22).

The labour required to process cassava depends on many factors(145, 146). Since cassava fields are normally some distance away there is a lot of walking and carrying of heavy loads of up to 30 kg(145). Combined with the work needed for processing the post-harvest production of cassava is labour intensive. This skewed labour division has been suggested to have a psychological effect between gender since the women are responsible for the whole chain of cassava from production to consumption(144)(p74-75).

## Processing techniques in Africa

Cassava processing techniques in Africa have been adapted from processing methods used for other crops that have traditionally been grown and eaten as staple food(59, 147). There is one method used for processing gari in West Africa that is reminiscent of the Amazonian Indians processing methods and this is no surprise since cassava was used as a provision on the ships navigating between the new world and Africa during the slave trade era(148). To get an understanding of the development of cassava processing in Africa one needs to look at practices related to indigenous African food crops such as yams(59), sorghum and millet (40). It has been suggested that the processing method for cassava in West Africa may stem from the techniques of processing toxic yams combined with the knowledge of the returnee slaves(139).

The processing of cassava in Africa may involve various combinations of the following steps: grating, soaking and fermenting, sun-drying, heap fermentation, leaching, roasting and steaming(62, 120, 147, 149-151). The chewing of the raw roots of “sweet” cassava cultivars has been described as a major thirst quencher and snack in much of East Africa (14, 67). This is not done with roots from “bitter” cassava cultivars. They are processed before consumption and the major products are mentioned below.

*Gari* is a product similar to *farinha de manioc* produced in Brazil. It entails peeling the roots, grating and pressing to dewater the mash. The mash can be left to dewater anywhere from one to several days. The mash is then roasted or fried in a pan(147, 149). Gari is commonly consumed in West Africa.

Soaking and fermenting roots followed by sun drying yields products known as *lafun* in Nigeria, *cossettes* in Congo(152). The roots are peeled, soaked in standing water or in earthenware pots for 1 to 4 days and fermented until soft. The drying of fermented roots can take 3 days or more and they are thereafter milled into flour. The soft root may also be crumbled and reshaped into balls before sun drying on mats or rocks to yield a product known as *kadonosya* in Malawi. Production of a *wet pulp* from soaked roots is common in Nigeria and Cameroon. The steps are similar to the soaking and fermenting method, however the mash is not sun dried but boiled in water and made into a thick paste. A similar method that yields a product called *chikwangue* is common to Congo. It is known by other names in other parts of Africa. It involves peeling, soaking and fermenting the roots for 3-5 days until soft. The fibres are removed and the pulp is further fermented in a heap covered with leaves and pressed with heavy objects. The mash is pounded to a finer texture, wrapped in leaves and steamed.

*Starch* is made when roots are peeled, washed, grated and steeped for 2-3 days in plenty of water stirred and filtered through a piece of cloth. The filtrate stands overnight and is decanted and air-dried. Simply by peeling, slicing roots into halves and thoroughly sun drying of roots on mats or roofs a product known as *makopa* is made in parts of Tanzania(153). The dried pieces can be stored and later be used to make flour. Heap-fermentation of peeled roots seems to have resulted out of necessity due to a shift from “sweet” cassava cultivars to “bitter” cassava cultivars in parts of East Africa(154).

In terms of effectiveness in reduction and removal of the cyanogenic glucosides and their breakdown products jointly know as cyanogens, the *soaking and fermentation* method seconded by *gari* have been found to be the most effective methods. *Soaking and fermentation* dictates that the roots ferment and become softened, sun dried thoroughly and pounded into flour. This ensures an almost total reduction of the total cyanogens up to 99.6%(89). The studies in this thesis were conducted in an area where the traditional method of cassava processing was *soaking and fermentation*. Studies from Nigeria have shown that *gari* processing can effectively reduce the total cyanogen content with 83% to 98% (127). Recent studies in Nigeria have found that cassava processors vary the length and procedures of gari processing to acquire the desired colour, taste and texture based on the preferred characteristics of the consumers. This has implications for the residual content of cyanogens in gari(155). Heap fermentation(154) is not as effective as the methods mentioned above and merely sun drying roots from “bitter” cassava cultivars is the least effective method for reducing the total cyanogens(156).

In times of food shortage it has been shown in some parts of Africa that women will shorten the processing sequence and apply methods that are less effective in reducing cyanogens. If the root cells are not disintegrated before drying large amounts of linamarin may remain. If roots are soaked for some days and pounded into flour before being dried thoroughly high levels of cyanohydrins may remain in the end product. As known by the farmers neither of these products from soaked cassava roots are safe for consumption and they are only consumed to avert hunger(152).

## CASSAVA AND HEALTH

Consumption of cassava products with residual amounts of cyanogens, mainly linamarin or acetone cyanohydrin, may result in dietary cyanide exposure. Ingested linamarin mainly passes through the body and is excreted unchanged in the urine. But if suitable glucosidases are available in the gut, either from the diet or the microflora, linamarin may break down and form cyanohydrin and thereafter cyanide. Ingested cyanohydrin will be stable in the low pH of the stomach but decompose in the higher pH of the gut. Cyanide is rapidly absorbed into the blood. Cyanide (CN) in the human body is mainly detoxified by an enzyme requiring sulphur as a substrate. The sulphur (S) is provided from sulphur amino acids to form the detoxification product thiocyanate (SCN). The latter is gradually excreted in the urine and urinary thiocyanate levels can be used for estimation of dietary cyanide exposure.

Some health problems and diseases have been attributed to dietary cyanogen exposure from consumption of cassava products with high levels of cyanogen residuals. High dietary cyanide exposure from one meal may rarely cause acute poisoning some hours after the meal(101, 156, 157). The symptoms usually pass within some hours but cassava poisoning may in rare instances be fatal. It is in fact peculiar that such acute poisonings are so rare considering the wide consumption of potentially toxic cassava roots.

Another condition that may be aggravated by dietary cyanide exposure is iodine deficiency disorders (IDD)(158-160). It is assumed that the detoxification product thiocyanate can interfere with the uptake of iodine by the thyroid gland. This effect only occurs when iodine intake is low and it can be quenched by iodisation of salt. Dietary cyanide exposure may also contribute to protein energy malnutrition due to the preferential use of sulphur amino acids for the detoxification of cyanide in the human body (161, 162).

The neurological disease konzo is characterised by sudden onset of a permanent spastic paralysis of both legs(163-166). It has been found to occur in rural cassava dominated communities in times of food shortage as a result of short-cuts in traditional processing methods (167). There is a strong geographical and temporal association between outbreaks of konzo and high dietary cyanide exposure from cassava. However, a causal role of cyanide has not been proven. Tropical ataxic neuropathy is another neurological disease that has mainly been described from Nigeria(165, 168). Its occurrence coincides with high consumption of the staple food *gari* with high cyanogen residuals. Unlike konzo it is not associated to food shortages(155) and the role of dietary cyanide in this disorder remains unclear.

In conclusion, these health problems attributed to cassava cyanogenesis are very rare in Africa in relation to the common use of “bitter” cassava cultivars as a staple food. It seems probable that the positive health effects of cassava mediated through improved food security for poor people in rural and urban areas are much greater than the accumulated possible negative effects of cyanogenesis. It therefore, constitutes a challenge to understand farmers’ perceptions about and strategies related to cassava cyanogenesis. Improved understanding of the farmers’ perspective could explain the rationale for the folk taxonomy into “sweet” and “bitter” cultivars. How does this affect the small-scale farmers’ selection of cultivars for adoption into the farming system. The studies in this thesis investigated the farmers’ preferences for “bitter” cassava cultivars having heard anecdotal statements that toxicity in cassava roots ameliorates food security.

# AIMS OF THE STUDIES

The overall aim was to elucidate the social and biological role of cyanogenesis in a cassava dominated farming system in Malawi where small-scale farmers employ the soaking method for processing roots from “bitter” cultivars.

The specific aims were:

1. To elucidate perceptions regarding taste and potential toxicity of cassava roots, the ethno-classification of cassava cultivars and the perceived rationale for growing cultivars with bitter and toxic roots (**Paper I**).
2. To estimate the relative importance attributed to each of the different reasons for growing cultivars with bitter and toxic roots (**Paper II**).
3. To assess the frequency of consumption of cassava products and the resulting dietary cyanogen exposure as measured by urinary levels of linamarin and thiocyanate (**Paper II**).
4. To elucidate to what degree cassava farmers can predict the levels of cyanogenic glucosides in raw cassava roots using their local knowledge about their cultivars, ethno-classification into “cool” and “bitter” cultivars and their tasting practices (**Paper III**).
5. To determine the correlation between levels of cyanogenic glucosides and the taste of raw cassava roots from different cultivars using a trained sensory taste panel (**Paper III**).
6. To assess to what degree each of the 10 most grown cultivars constitute one single or a mixture of genotypes (**Paper IV**).
7. To determine the accuracy with which farmers are able to identify plants as belonging to a specific cultivar(**Paper IV**).

# STUDY AREA

Malawi (figure 1) has approximately 10 million inhabitants with 47% of the population concentrated in the southern region and the remaining 41% and 12% in the central and northern regions, respectively(169). The sex distribution differs in the rural and urban area with more males in the urban area. Half of the population is below the age of 18 years and 4% are 65 years or older. Out of the economically active population 83% are involved in agriculture and 73% are subsistence farmers producing almost all the food they eat and only having very small landholdings(170). Literacy rates are 64% in males and 52% in women. The northern region has the highest literacy rate, 72%. Maize is the most important staple crop in Malawi followed by cassava(56). The official languages in Malawi are Chichewa and English, with Chitumbuka as the lingua franca in the northern region. Of the total population 80% are Christian and 13% are Moslems(169).

Malawi is severely affected by HIV/AIDS. The prevalence of HIV infections among adults is estimated at 25% in urban and 12% in rural areas (<http://www.undp.org/hiv/malawi.pdf>). Half a million children and teenagers have only one parent alive, usually a mother. Infant mortality rate is 121/1000. It is estimated that 66% of the population live in dwellings with thatched roofs and walls made of mud. More than 90% of the population use firewood as their main source of energy for cooking. According to The World Bank statistical data base Malawi has a GNP of 190 USD(171) and a recent survey by the Ministry of Economic Planning and The International Food Policy Research Institute (IFPRI) found that the number of people living below the poverty line was 65% in 1998. Given this high level of poverty Malawi desegregates the percentage of households considered to be *ultra poor*, that is households with consumption levels less than 60% of the poverty line. The national figure for the *ultra poor* is 28%, with the rural area having the highest percentage(172).

## NKHATA-BAY DISTRICT

All the four studies were conducted in Nkhata-Bay district located in the northern region. Nkhata-Bay has a population of approximately 165,000 persons with a density of about 40 persons/km<sup>2</sup>(169). The two islands in Lake Malawi belong to the district at the time of the study but have now been granted district status. Likoma Island is now the most densely populated district in the country 449 persons/km<sup>2</sup>. In addition, the islands have the highest percentage (16%) of persons below the age of 20 with both parents' deceased. The poverty level for the district is estimated at 55% and the average number of persons per household is 4.9(169).

Nkhata-Bay is primarily an agricultural district with a small fishing economy along the lakeshore mostly producing for domestic use. The main staple crop in this area is cassava(61). The main dish made from cassava is called *kondowole*(119, 173), a dumpling-like product that is eaten together with a sauce made of cassava leaves, fish or other foods using fingers. The main ethnic groups are the Tonga with minor groups of the Tumbuka people and to an even minor extent the Chewas. A rather high proportion of households are female-headed due to widowhood, migration of the male for labour employment in the South of Africa or due to being abandoned by the husband. The population has a patrilineal heritage and polygyn is practised.

### *Study area and subjects of Paper I*

Nkhata-Bay district is divided into four agro-ecological zones; 1) the islands, 2) the lakeshore zone at 475-600 m above sea level, 3) the escarpment zone 600-900 m and 4) the mountainous and plateau zone at more than 900 m above sea-level(174). Based on the Ministry of Agriculture Research and Extension the district is divided into four extension planning areas: Nkhata-bay, Mpamba, Chintheche and Chitheka(figure 1). Chitheka was excluded from the study due to accessibility problems. The remaining three extension areas were administratively divided into 13, 13 and 14 sections, respecti-

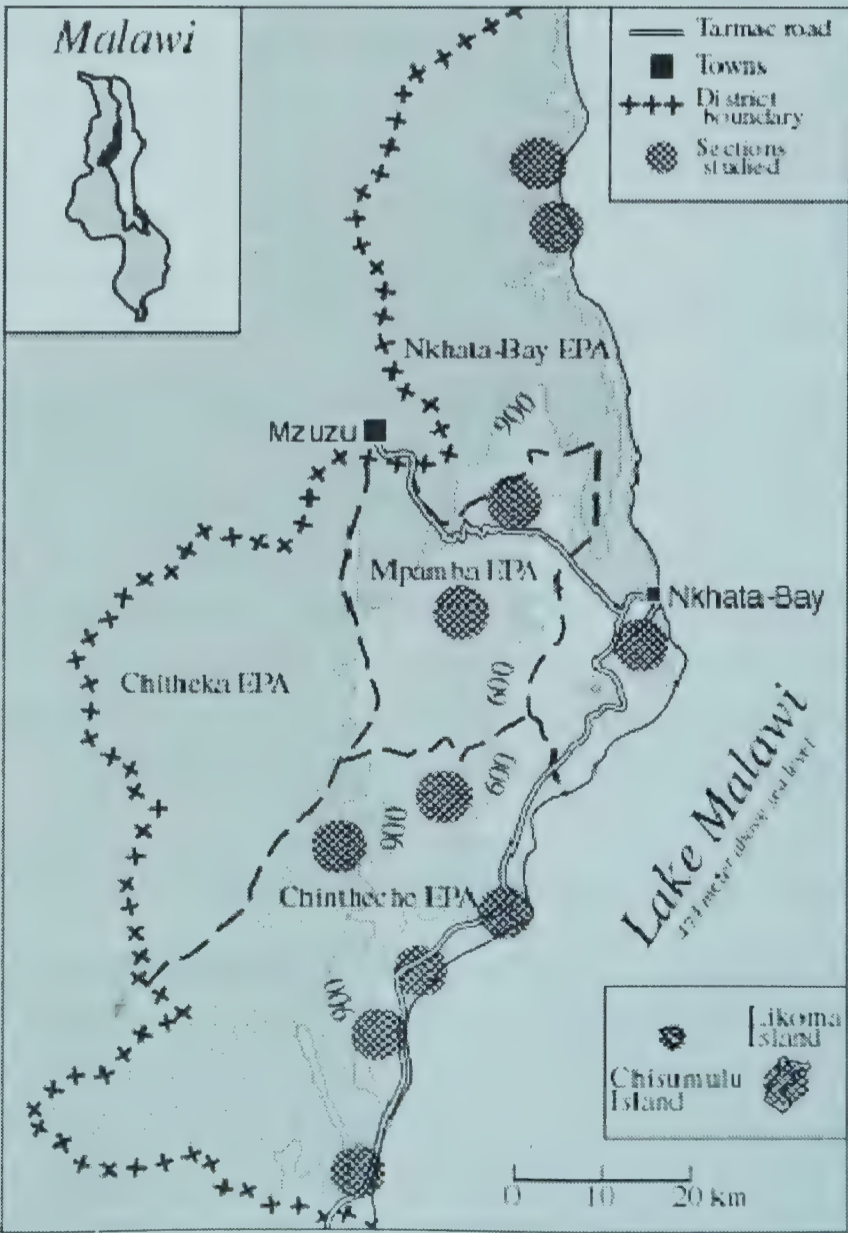
vely, and a Field Assistant (FA) headed each section.

A total of 13 sections out of the 40 were included in the study and they were chosen to represent maximum agro-ecological variation. The interviewed subjects were selected to achieve maximum heterogeneity. They came from both male and female-headed households with land holdings from as little as 0.2 ha. Women were preferentially selected since women predominantly conduct cassava processing. A total of 43 key-informants comprising male agricultural extension staff, chiefs, notable male and female farmers and knowledgeable elderly women and men from the community were interviewed. Twelve persons were interviewed in-depth, 10 focus group interviews were conducted and participant observations were conducted to elucidate the practices of cassava farming in all the 13 sections visited.

*Study area and subjects of Papers II, III and IV*

Chintheche Extension Planning Area (EPA) was selected for these studies because of being regarded as representative of the densely populated lakeshore area and due to infrastructural proximity to the laboratory facilities at Mkondezi Agricultural Research located 50 km north of his area. Out of the 13 sections in the area, Lweya and Mgodi, were included in the surveys as they were perceived to be representative of the EPA-area. A house to house survey identified 98 households in Lweya and 102 in Mgodi. One block from each section was selected to represent the social and agricultural variation in the area. The two blocks, “Matyenda 1” and “Thowolo-B” were adjacent to each other. Only the women in charge of cooking in the households were interviewed. Those households where the woman was missing, or where no urine sample was obtained were ultimately excluded from the study ending up with 176 households (*Paper II*). In *Papers III and IV* a sub-set of 30 farmers were consecutively selected from the 176 based on the presence of the woman in charge of cooking and handling of cassava. Two women were excluded due to failure to extract adequate DNA samples from the plant samples. *Paper III and IV* eventually had 28 farmers included in the studies.

**Figure 1. Map of Nkhata-Bay district showing Extension Planning Areas (EPA) for agriculture and the 13 sites of study I.**



# MATERIALS AND METHODS

## QUALITATIVE INTERVIEWS (PAPER I)

A pilot survey was conducted during the months of November and December in 1993 in Chitipa district. The pilot study was conducted to assess the feasibility of elucidating the role of cassava cyanogenesis in Malawi. The findings revealed that soaking was the preferred method of processing and that farmers possessed a wealth of information regarding cassava classification, farming, processing and consumption. This was the foundation of the four studies.

The main fieldwork was conducted during the dry season in September and October in 1994, and a complementary survey was conducted in the same season in 1995. Since this was an exploratory study to gain an insight in the possible reasons for use of “bitter” cassava the selection of subjects was conducted according to purposeful selection(175) based on a fulfilment of a criterion that would lead us to knowing more about the farming of “bitter” cassava. All the interviews were done in the local languages Tonga, Tumbuka and Chichewa, and a few in English when the interviewees so preferred. The main investigator spoke three of these languages fluently and comprehended a reasonable level of Tonga.

### *Key-informant interviews*

Persons perceived as being able to provide the researchers with the initial information were interviewed as key-informants(176, 177). The informants were asked to recount their experiences with cassava farming in the area and a short checklist was used to guide the narratives. These informants also gave names of persons and locations from where we could obtain the information to best answer our research questions.

### *Structured direct observations*

In all 13 sections structured direct observations(178) were done on cassava farming, harvesting, processing, storage, preparation and consumption. Markets were visited to get a perspective of sales of cassava in the area. These observations lasted from a day to a week depending on the process of observation.

### *In-depth interviews*

Following observations of procedures pertaining to cassava farming individual interaction was sought to get information on particular issues. Interview discussions(177) were held to get answers on the what, why and how questions vis-à-vis: cultivars grown, taste, toxicity, method of planting, processing whole roots in earthenware pots versus in shallow pools, drying whole roots, small pieces or specially designed shapes (*kadonosya*), pounding manually or milling the dried roots at a mill. A total of 12 in-depth interviews were conducted.

### *Focus group discussions*

After gaining insight into the reasons for farming “bitter” cassava cultivars focus group discussions were conducted specifically on this topic. A total of 10 focus group(176, 179-181) sessions were conducted to get a range of ideas, reasons, and confirmation of the perceived reasons. We conducted mixed gender discussions and when an idea was silenced it was followed up with an in-depth interview(182). The focus group discussions were interactively used to elucidate the range of knowledge, practices, attitudes and beliefs related to cassava cultivation, processing and consumption and preferences for cassava cultivars. Field notes from all discussions, interviews and observations as well as 12 audio-recorded and six video-recorded interviews were transcribed into English and classified into salient themes according to content.

## QUANTITATIVE AND SEMI-STRUCTURED INTERVIEWS (PAPER II)

A questionnaire formulated from the results of the qualitative interviews(183) in Paper I was used in all the 176 households in the two selected sections that were interviewed by the author together with two male assistants in July and August, 1996. The interviews included both closed and open-ended questions and included bio-social data, names of cassava cultivars grown and classification of these cultivars into the group of “bitter” and “cool”, respectively. Advantages and disadvantages in their cassava farming of “bitter” and “cool” cultivars were systematically asked for.

In the interviews each woman was also asked about the frequency of consumption of cassava and other food products during the past 7 days(184-186). There were five cassava products of major interest and a total of 17 food items were included in the questionnaire. The frequency options were three, twice or once daily, four to six or three times per week; or never. There was deliberate decision to have as few choices as possible biased towards the higher frequency since lower frequency has been shown to contribute very little to nutrient intake(187).

### *Dietary cyanogen exposure estimated by urinary thiocyanate excretion*

Dietary cyanogen intake occurs when cassava products that contain residual cyanogens are consumed(167). This intake can be estimated from urinary levels of thiocyanate and linamarin. Thiocyanate is the main (70-80%) cyanide detoxification product in well nourished humans and the elimination half-life in healthy humans is estimated to be 3-7 days(188). Linamarin may pass through the body unchanged(189) or it may be broken down in the gut where it releases cyanide (CN) that after absorption into the blood is converted to thiocyanate (SCN) in the tissues and then excreted in the urine(156, 190). Urine samples, 20 ml, were collected from 176 women on the day of the interview. The samples were kept in a cool box and frozen at  $-20^{\circ}\text{C}$  at the end of the interviews conducted on that day. The samples were transported in a frozen state to Sweden in a cool box with packed ice where they were analysed for linamarin(190) and thiocyanate(191).

## SAMPLING AND TASTING OF ROOTS (PAPER III)

### *Survey and interviews*

In September 1996 a sub-set of 28 consecutively selected women from the 176 women interviewed in Paper II were included on the basis of growing and having ready to harvest cassava roots of one or more of the ten most commonly grown cultivars. Women identified in their own fields two plants of the same cultivar and predicted the expected danger level into five categories from not dangerous to very dangerous. In predicting taste at home they used a six-grade scale as follows: *very cool* (1), *cool* (2), *intermediate* (3), *bitter* (4), *very bitter* (5) and *very very bitter* (6). The predictions about taste of roots from each cultivar were also done at the edge of the field before identifying the plants. During analysis grades five and six were merged as one grade five. The farmers thereafter tasted the two roots harvested from each plant and assessed the taste using the above five taste scores. Tasting of the roots was done away from the field and the women only tasted the tip of the root in the regular way they use to do.

A total of 492 roots were sampled from 246 plants from the ten most grown cultivars. Of the 492 roots 48 were ‘nyamaokozo’; 56 ‘gomani’; 52 ‘nyankhata’, 52 ‘ngwenyani’; 52 ‘depweti’; 42 ‘nyaharawa’ 52 ‘koloweki’ 44 ‘nyachikundi’; 40 ‘chimpuno’; and 48 ‘mbundumali’, respectively. The farmers classified the first seven cultivars as “bitter” and the latter three as being “cool”. Once the farmers had tasted the roots, the roots were bagged and transported to Mkondezi Agricultural Research Station. At the laboratory, the roots were peeled within one hour, washed under running tap water and split longitudinally. The roots were bagged in plastic bags and one half was directly used for the chemical analysis while the other was simultaneously used in a separate room for the sensory analysis by the taste panel. The roots were cut into 2 – 3 cm pieces for the taste panel.

### *Taste panel*

At Mkondezi Agricultural Research Station, thirty self-reportedly healthy, non-smoking, non-habitual cassava consumers with at least eight years of education were screened for the taste panel in 1995 and 1996. Following standard procedures(192, 193) the volunteers were assessed for their threshold levels for the bitter tasting compound caffeine based on the lowest level (0.6 mg caffeine/l water). Four panel selection sessions were conducted and each prospective panellist was assessed twice. The “tasters” (sensitive) were separated from the “non-taster” (insensitive) persons. The 15 assessors that were able to identify the lowest concentrations of caffeine in solution ranging from the blank samples (distilled water) to the highest levels were selected for the taste panel.

Considering that human perceptions and interpretation of taste is a complex process(194) every possible attempt was made to standardise. The selected panellists were served lunch everyday (11 days) during the study period, a short interval was included halfway through the session and daily discussions were held with the assessors to get their views on the whole process. The longitudinally split 492 root-halves were cut in sizeable pieces between 2 –3 cm and served to the panel. A total of 48 - 50 samples were tasted each day. A mean taste score was calculated on the five-category scale given by the 12-member panel for each root.

## **CYANOGEN GLUCOSIDE DETERMINATION IN RAW CASSAVA ROOTS**

Cyanogenic glucoside determination was conducted on 492 root halves. The root was cut into approximately one cm sized cubes. An amount of 49.5 -50.5 g was weighed into a plastic cup and mixed with 160 ml 0.1 M orthophosphoric acid and mixed in a blender according to the method of Brimer(195). Total cyanogens were determined by a micro-diffusion method using solid-phase detection described by Saka(196). Moisture content of each root was determined by oven drying of cubes until constant weight was attained.

## **QUALITY CONTROL OF TASTE AND CHEMICAL ASSAYS**

The reproducibility of the chemical analysis and the taste assessments were calibrated with double determinations of 11 roots. Each of these roots was divided into four equal longitudinal portions to obtain double determinations for both mean taste-scores and the levels of cyanogenic glucosides. The investigators conducting the taste panel and the chemical analysis were blinded for the doubling of these 11 samples. The eleven double estimations of mean taste score yielded a correlation coefficient of 0.95 and those for chemical determination 0.99.

## **FARMER IDENTIFICATIONS, PLANT MORPHOLOGY AND MOLECULAR GENETICS (PAPER IV)**

### *Farmers' identification*

In September 1996 the same sub-set of 28 women interviewed in Study III were asked to identify two plants belonging to one of the ten cultivars that they claimed as having in their fields. They did this by freely walking through their fields and observing as many plants as they wanted. The same plants used to obtain roots for study III were used to obtain material for DNA analysis.

### *Morphological cassava plant description*

A total of 246 plants identified by the farmers as belonging to one of the ten most grown cultivars were tagged with an identification number and morphologically described using a modified botanical identification key from the COSCA studies(53). Morphological characterisation of above and below ground parts of each plant was conducted and assessed: shoot pubescence, shoot colour, leaf colour, leaf shape, petiole colour, leaf lamina colour, mature stem colour, root neck length, outer root skin colour, inner root skin colour and root pulp colour. After the examination, and with due permission

from the Ministry of Agriculture of Malawi, the cuttings were transported to and re-planted in a greenhouse at the Swedish University of Agricultural Sciences, Uppsala, Sweden.

### *Short sequence repeat (SSR-markers) analysis*

Following leaf development after about one month, DNA was extracted from two fresh unexpanded young leaves of each plant(197). All DNA samples were treated with RNase. Out of 123 collected pairs of plants, sufficient quantity of DNA was extracted from 116 pairs and these 232 plants were included in the study. Seven short sequence repeat (SSR) primer pairs(198) were used in multiplex polymerase chain reaction (PCR) amplifications to identify the genotype make-up of the plants. Furthermore, stem cuttings were collected in 1997 from 45 plants identified by farmers as belonging to a cultivar that was not among the ten most grown studied in 1996. All the stem-cuttings were transported to Sweden for molecular marker analysis with permission as described above and method as described above.

## **ETHICAL CONSIDERATION**

The studies were approved by the Research Division and Extension Service of the Ministry of Agriculture, the District Health Commissioner in Nkhata-Bay, and by oral informed consent from the community leaders and all participating farmers.

## **STATISTICAL ANALYSIS**

Simple descriptive statistics, means, confidence interval, Anova and regression using SPSS® version 9.0 and 10.0 were used to analyse the data in **Studies II, III**. In **Study IV** Fishers exact test and chi-square test was used to compare proportions. Principal component analysis was done on the SSR allelic data. A multivariate fit (JMP® 1994, Multivariate fitting) was done on 7 of the 11 morphological variables of the 181 plants having any of the ten genotypes. Morphological variables that did not vary were excluded.



Fig 2a. Left to right: James Ngoma, FA Botha & Sidney Simukoko interviewing



Fig 2b. The Lunch Team and Drama Leader



Fig 2c. Taste Panel members in action



Fig 2d. Taste Panellists taking a 30 minute break

Figure 2. Photos from different aspects of the studies

# SUMMARY OF RESULTS

## PAPER I

In this qualitative study, farmers reported that cassava was not only the preferred staple food but that it had been like this in the area as long as the oldest respondent could remember (91 years old). All those interviewed stated that the “bitter” cultivars were preferred for the making of the staple food *kondowole*. Although other crops such as maize and rice were also grown they strictly remained the preference of the minority ethnic groups whereas for the Tongas, the destruction of the cassava crop by the mealy bug in 1986–90 was what made them start eating maize. Since water was readily available in this area the preferred method of processing was soaking and fermenting for 2 to 5 days, followed by sun drying and pounding into flour. The flour was used to make the staple food *kondowole*. This method of processing was used for roots of both “bitter” and “cool” cultivars to acquire the preferred end products. The women reiterated that processing was not perceived as a problem whereas the last step of processing i.e. pounding the dried roots into flour was a painstaking effort. Mechanical mills were therefore strongly wished for by the women to ease their daily work.

Farmers had specific local names for each of the 54 named cultivars that were reportedly grown in the district. In addition to the names of each cultivar farmers also used a classification system for the cultivars that denoted whether roots from a particular cultivar need elaborate processing prior to consumption or if the roots could be eaten directly without any processing. The classification system grouped all the cultivars into either of two groups, *vyakubaba* synonymous with “bitter” and *vyakuzizira* literary meaning “cool” cassava. The naming of the cultivars were related to origin, provenance (people, villages or names) or peculiar characteristic specific to the cultivar but did not indicate if the cultivar was “bitter” or “cool”. Out of the 54 name-given cultivars, 36 were classified as *vyakubaba* and 18 were classified as *vyakuzizira*. The farmers stated that they also had some cultivars that were classified as intermediate *vyakubabira*, and these usually happened to be those cultivars that had roots that shifted in taste from either one of the two aforementioned groups.

There was a consensus among all the interviewed farmers that bitter taste in cassava roots predicted the potential toxicity of cassava roots if consumed without any prior processing. Hence taste of raw roots was perceived as predicting the need for processing. The prediction of taste was scored on a continuum starting from *very cool*, *cool*, *intermediate*, *bitter* to *very bitter*. Farmers deemed it sufficient to taste the tip of the root for bitterness and this process was known as *kucheta*. Farmers stated that they did not classify cassava as “sweet” because the roots did not taste like sugar, they either tasted good or bad, that is *chizika* or *chibaba*. Cassava roots that did not fall into any of these categories were killed i.e. taken away from the farming system.

Most importantly, destitute women and men had a very high preference for “bitter” cassava cultivars (table 4) because the need to process indirectly protected their crop from thievery. This preference for “bitter” cassava cultivars that the farmers associated with food security was locally known in Tonga as *kuvikiliya*, it literary means to store in the ground. The farmers perceived the poison in cassava to be an important deterrent to both humans and vermin. Furthermore, farmers strongly associated high yields with “bitter” cassava. The overall protection given by the toxin was the main determinant for the preference of bitter cassava but the desired end products were also perceived as being of a superior quality when produced from “bitter” cassava. The two products perceived as being better when made from “bitter” cassava were the leaves and flour.

## PAPER II

A total of 176 women were included in the study out of a total of 200 households identified in the census. The range of the women’s age was 15 to 70 years and the mean age was  $40 \pm 15$  years. The majority of the women were married (55%), engaged in agriculture and the proportion (72%) of

women that were heads of households increased with age i.e.  $\geq 50$  years. The mean household size was  $5.5 \pm 0.2$  and the largest household had 13 members.

**Table 4. Identified preference for “bitter” cassava cultivars**

Groups of reasons	Specific reason
<i>Protection perception</i>	<ul style="list-style-type: none"><li>• Deters human theft</li><li>• Curbs unplanned harvest and social obligations</li><li>• Deterring animal destruction</li></ul>
<i>High yield</i>	<ul style="list-style-type: none"><li>• Yields well in poor soils</li></ul>
<i>Superior end-product quality</i>	<ul style="list-style-type: none"><li>• Flour whiter and more compact</li><li>• <i>Kondowole</i> less stringy</li><li>• <i>Vipumu</i> (soaked &amp; roasted roots)</li><li>• Leaf relish tastier and tender</li></ul>

The women interviewed in the two communities stated that they grew 45 name-given cultivars that were classified as belonging to either “cool” or “bitter” cassava. However, a total of 61 cultivars were reportedly grown and 16 of them were identified as an unnamed cultivar. Out of the total 43 were classified as being “bitter” and 18 of them were classified as “cool”. Of all the cultivars grown in the area, the cultivar ‘gomani’ (69%) and ‘depweti’ (64%) were grown by the highest percentage of women and they were classified as “bitter” cultivars. The “cool” cultivar ‘mbundumali’ was grown by 52%. Only 5 women (3%) did not cultivate any cassava and the mean number of cultivars grown by each woman was  $4.6 \pm 2.4$ .

The semi-structured interviews showed that protection against unplanned harvesting, theft and animal destruction were the most important reasons for growing “bitter” cassava, 91%, 90% and 74%, respectively.

Farmers were asked to score the expected average taste of cassava roots using six categories that ranged from *very cool* (1) to *very very bitter* (6). For the cultivars designated as “cool” the mean taste scores ranged from 1.0 to 1.7, while those designated as “bitter” ranged from 3.8 to 5.0. The scoring of the danger of eating fresh roots ranged from no danger (1) to very dangerous (5) and for those cultivars designated as “cool” the mean danger risk ranged from 1.0 to 1.3 where as the “bitter” cultivars ranged from 2.4 to 3.8 (table 5). The farmers scoring of danger and taste for the 45 cultivars grown by more than one farmer showed a strong correlation ( $r > 0.98$ ). The scoring of the cultivars divided the cultivars into two groups, one clearly clustering as “cool” and not dangerous and the other as “bitter” and dangerous.

A total of 55% of the women reported consuming kondowole on a daily basis during the past seven days. This number was reported as being low because there had been heavy rains that year in the area and many farmers had lost their cassava crop. At the time of the interview many of the plants only had immature roots. A higher proportion of women-headed households consumed cassava more frequently than their counterparts and the overall mean age was higher for these women. Three of the women admitted to experiencing acute cassava poisoning and attributed the incidents to lack of time for effective processing of the roots.

Urinary thiocyanate concentrations ranged from 2 to 410  $\mu\text{mol/l}$  and the median was 32  $\mu\text{mol/l}$ . Linamarin levels were lower (table 6). Only 23 women had thiocyanate levels above 100  $\mu\text{mol/l}$ . The three women that had admitted to acute effects of ineffective processing had thiocyanate levels of 60, 118 and 410  $\mu\text{mol/l}$ , respectively. The corresponding linamarin levels were, 7, 24 and 7  $\mu\text{mol/l}$ ,

respectively. The woman with the highest levels had recently emigrated from Mocambique where her traditional practice had been sun drying and not soaking. No correlation was found between urinary thiocyanate and linamarin concentration.

Total cassava consumption was calculated as the total sum of meals of soaked and roasted, raw, boiled, roasted or *kondowole*. There was a significant difference (t-test) between the mean urinary thiocyanate concentration among the 142 women having eaten kondowole during the last week (table 6) compared to those not having consumed kondowole during the past seven days ( $p < 0.01$ ). There was no similar association found with the consumption of other foods.

Table 5. The mean ±SEM of danger and taste scoring for average roots for each cultivar grown in the farmers' own fields

Type and name	Number of women growing	Mean ±SEM danger score	Mean ±SEM taste score
<b>“Bitter”</b>			
Gomani	122	3.5 ±0.1	4.7 ±0.1
Depweti	113	3.2±0.1	4.4 ±0.1
Koloweki	76	2.7 ±0.1	4.1 ±0.1
NyaHarawa	52	2.9 ±0.1	4.3 ±0.1
NyaNkhata	37	3.5 ±0.1	4.6 ±0.1
Ng’wenyani	27	3.5 ±0.1	4.7 ±0.1
Nyamakozo	23	3.7 ±0.1	4.8 ±0.1
Mpuma	20	3.1 ±0.3	3.9 ±0.4
Palamu	20	3.8 ±0.1	4.9 ±0.1
Chakubaba	16	3.6 ±0.2	4.7 ±0.1
Kanonono	13	2.4 ±0.2	3.8 ±0.2
Munyakayuni	11	3.5 ±0.3	4.7 ±0.2
Kawalika	10	3.6 ±0.4	4.6 ±0.4
Mzumara	10	3.6 ±0.2	5.0 ±0.0
Thipula	8	3.5 ±0.3	4.4 ±0.3
Nyasungwi	7	3.6 ±0.3	4.7 ±0.2
Mbayani	6	3.0 ±0.5	4.3 ±0.3
<b>“Cool”</b>			
Mbundumali	92	1.0 ±0.0	1.0 ±0.0
Chimpuno	31	1.0 ±0.0	1.1 ±0.1
NyaChikundi	31	1.0 ±0.0	1.1 ±0.1
Fyoka	15	1.1 ±0.1	1.3 ±0.2
Kweti	7	1.3 ±0.3	1.7 ±0.5
Mwaya	3	1.0 ±0.0	1.0 ±0.0
Kamwala	2	1.0 ±0.0	1.0 ±0.0
Katchamba	2	1.0 ±0.0	1.0 ±0.0

Table 6. Urinary thiocyanate and linamarin levels in women with different cassava consumption frequency

	Kondowole consumption frequency			
	Daily	Weekly	Never	Total
	N = 98	N = 44	N = 33	N = 175
Head of household	47 (48%)a	10 (23%)b	10 (30%) c	67 (38%)
Thiocyanate mmol/l	57 (±7) a	48 (±6) a	29 (±5) b	50 (±4)
Linamarin mmol/l	14 (±1) a	13 (±1) a	13 (±1) a	14 (±1)

Means followed by different letter are significantly different from each other by student's t-test and Chi-square, respectively (P < 0.01).

12300  
Ae12-100  
201

## PAPER III

The mean  $\pm$ SEM levels of cyanogenic glucosides expressed as HCN eq kg<sup>-1</sup> fresh root were in roots from 'nyamakozo' 242 $\pm$ 2; 'gomani' 161 $\pm$ 1; 'nyankhata' 160 $\pm$ 1; 'depweti' 120 $\pm$ 7; 'nyaharawa' 117 $\pm$ 9; and 'koloweki' 114 $\pm$ 7 mg, respectively. The three "cool" cultivars had; 'nyachikundi' 31 $\pm$ 4; 'chimpuno' 30 $\pm$ 4; and 25 $\pm$ 3, respectively. The mean glucoside levels for the 132 roots from the "cool" cultivars was 29 mg HCN eq kg<sup>-1</sup> (confidence interval 25-33 range 1 – 123) and the 360 roots from the "bitter" cultivars had a mean of 153 mg HCN eq kg<sup>-1</sup> (confidence interval 143 - 163 range 22 – 661). There was an overlap in glucoside levels between the roots that are classified as coming from the "cool" and "bitter" cultivars (figure 3). There is a continuum of glucoside levels in the roots from both the "cool" and "bitter" cultivars ranging from 1 – 661 HCN eq kg<sup>-1</sup> fresh weight, whereas the means of roots from the different cultivars clearly falls into two groups, "cool" and "bitter", respectively.

There was a strong correlation (0.87) between the cyanogenic glucoside levels (log scale) of the total 492 roots and the mean taste score by the sensory panel. There was a large dispersion of cyanogenic glucoside levels occurring between the mean taste scores of 4.5 and 5.0, i.e. in roots observed as being assessed as having a very bitter taste by most of the panellists (figure 3).

The farmers' general predictions of taste for each cultivar expressed as mean predicted taste score for each cultivar was statistically significantly correlated ( $r = 0.82$ ) with the mean glucoside level for roots of each of the seven bitter cultivars. When analysing the correlation at the root level already farmers' classification into "bitter" and "cool" cultivars gave a correlation coefficient of 0.56 with the cyanogenic glucoside levels. General prediction about each cultivar at home compared with cyanogenic potential of roots improved the correlation to  $r = 0.61$ . This improved further to 0.65 when comparing farmers taste score after tasting the tip of the root with the glucoside levels.

## PAPER IV

The specific allele pattern that was found in the eight SSR loci comprised the genotype of each plant. The single genotype found in the majority of the plants identified as belonging to one cultivar was referred to as "typical" for that specific cultivar. Those plants that did not display any of these genotypes were referred to as having a "non-typical" genotype. There was one single genotype found in the majority of the plants (54 – 100%) identified as belonging to each of the ten most commonly grown cultivars. Out of the 181 plants with "typical" genotypes, the farmers mis-classified 14 plants as belonging to the wrong cultivar as shown in table 7. Most of the plants with "non-typical" genotypes that were identified by the farmers as belonging to the same cultivar had different "non-typical" genotypes. There was a total of 29 "non-typical" genotypes found.

The mean cyanogenic glucoside levels expressed as HCN eq kg<sup>-1</sup> fresh weight were three to ten-fold higher in plants with "typical" genotypes of "bitter" cultivars than in the plants with "typical" genotypes of "cool" cultivars. This pattern was the same also for the plants with different "non-typical" genotypes of "bitter" and "cool" cultivars, respectively.

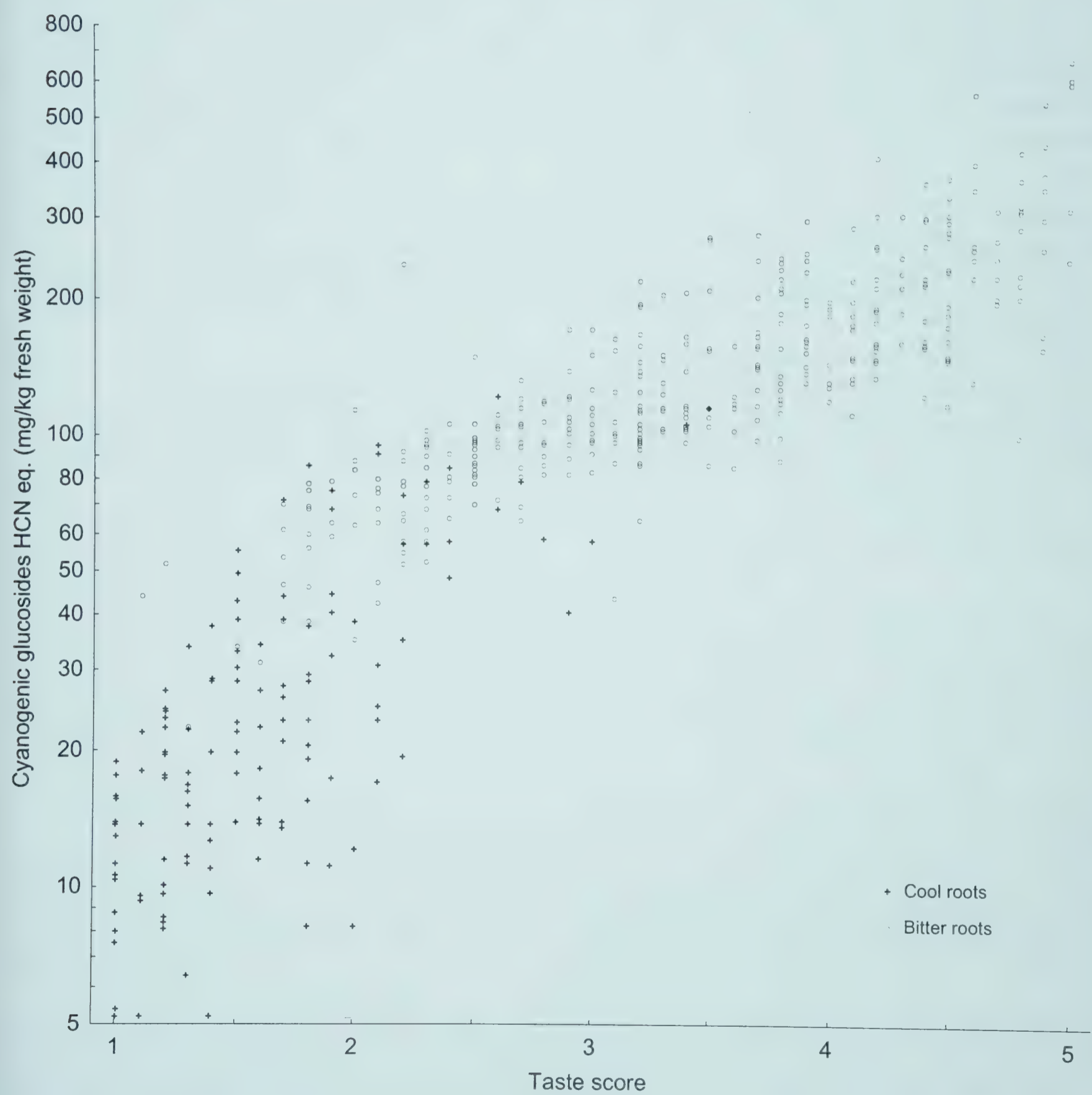
The highest agreement between what the farmers said and the molecular marker identification of the plants were for the cultivar 'gomani' (100%), 'mbundumali' (92%) and 'nyamakozo' (88%). Of the total 232 plants, the farmers classified 170 plants as belonging to the "bitter" and 62 as "cool" cultivars.

The farmers in the two communities differed in their ability to classify the plants, as belonging to a particular cultivar and the variation was very cultivar specific, for example the cultivar 'depwete', 'nyankhata' and 'nyamakozo'. The community with the lowest number of cultivars per household, Matyenda-1, had a mean of 3.6 cultivars per household. They more often had plants with "typical" genotypes correctly identified.

Since there were no differences observed on leaf shape and colour, inner root skin and colour of root pulp this data was excluded. Discriminate analysis was conducted using the remaining seven morphological

hological characters observed in the 181 plants with “typical” genotypes. The discriminate analysis of the morphological description by the investigators of the plants revealed a considerable overlap between all the cultivars except for the “cool” cultivar ‘mbundumali’. Despite this the farmers unlike the scientists were adept at separating the plants and correctly identified the “typical” genotype. The “bitter” cultivars with “typical” genotypes not only overlapped with the “cool” but two of the “bitter” cultivars ‘gomani’ and ‘nyamakozo’ were almost inseparable and yet farmers could separate all plants with typical genotypes of these two cultivars.

A principle component plot of the “typical” genotypes on the SSR allele composition of the ten genotypes revealed a significant difference ( $p < 0.001$ ) between the allele composition of the two groups of cultivars “bitter” and “cool”. The genotypes of the three “cool” were genetically separated from those of the seven “bitter” cultivars. When all the allele-pattern of the 39 “typical” and “non-typical” genotypes found in either “cool” or “bitter” cultivars were included they also clustered into two significantly different groups ( $p < 0.001$ ).



**Figure 3.** Correlation of cyanogenic glucosides HCN eq kg<sup>-1</sup> fresh weight (logarithmic) and the mean taste score as determined by the sensory panel for each of the 492 roots

Table 7. Agreement between farmers' identification of cultivar and laboratory identification of genotypes of 232 plants

Cultivars <sup>1,2</sup>	No. of plants	Number of plants with genotypes that are:										Agree-ment <sup>3</sup>		
		typical											non-typical	
	Mb	Ch	Nc	Go	De	Ko	Nh	Nk	Ng	Nm		cool	bitter	
Cool														
Mbundumali(Mb)	24	22										2	92	
Chimpuno (Ch)	18		13									5	72	
NyaChikundi(Nc)	20			15								5	75	
Bitter														
Gomani (Go)	22				22								0	100
Depweti (De)	26					19				1			6	73
Koloweki (Ko)	24					5	14						5	58
NyaHarawa (Nh)	24				1	1		13	1				8	54
NyaNkhata (Nk)	26				1	1			15		1		8	58
Ng'wenyani (Ng)	24					2				13			9	54
Nyamakozo (Nm)	24										21		3	88
Total	232	22	13	15	24	28	14	13	16	14	22	12	39	
HCN mean <sup>4</sup>		26	25	32	163	111	123	112	203	177	251	40	119	
HCN SE <sup>4</sup>		4	7	7	17	7	11	16	19	28	26	26	4	

1. Within “cool” and “bitter” groups, cultivars are listed in descending order according to the proportion of farmers in the area growing the cultivar

2. The abbreviation in parenthesis designates the “typical” genotype of each cultivar.

3. Percentage of plants with “typical” genotype among plants identified as belonging to each cultivar.

4. Mean and SE of the level of cyanogenic glucosides in root parenchyma of all plants belonging to each “typical” genotype and plants with “non-typical” genotypes among “cool” and “bitter” cultivars, respectively, expressed as mg HCN equivalents per kg fresh weight

## DISCUSSION

These studies were conducted in Nkhata-Bay district where cassava has been the number one staple food for over a century(107, 108, 199) and where more than 70% of farmed land is occupied by cassava. A proverb in *Tonga*, the predominant language in the district summarises the importance of cassava. *Chigawo chilera balanda, kuchanya nidendi, pakati ni mbeu na nkhuni, pasi ni sima*. The literal translation is, *cassava nurtures the destitute, the top is the relish, the middle is both planting material and firewood and the bottom is the staple food sima*. Cassava is the “main staff of life” in this area and to have a field without cassava is synonymous with putting one’s survival in danger. The studied communities have accumulated knowledge about cassava during more than five generations.

As early as the 1960ies it was concluded that root crops such as cassava would play a major role in increasing food production in Sub-Saharan Africa(200, 201) and this prediction has proven to be true(55). Throughout Malawi cassava production is on the increase(56). In the area where the studies were conducted the contemporary change in farming is due to increasing population density. Landholdings are diminishing, a process referred to as “land fragmentation”, and fallow periods has become almost non-existent. Another characteristic is that markets are yet to be well organised(61). The situation of this area is very similar to that of other cassava growing areas in the region. Therefore, these findings are not peculiar to the studied area but they may be extrapolated to many parts of Eastern and Southern Africa.

The term *farmer* in the discussion primarily refers to a *female farmer*. Women in this area predominantly carry out cassava production and processing. Men spend most of their time fishing or they are engaged in other crops that have higher economic potential such as tobacco. What is most interesting is the proportion of “bitter” cassava that is grown and why. The main reasons given by the farmers for why “bitter” cassava was deemed more important in their farming system was the fact that roots of these cultivars are not prone to thievery when left standing in the fields in a state of ready for harvest. The reason why “bitter” and toxic roots are not readily stolen is that they require immediate processing lasting more than five days before providing a safe food. This reason alone deters those that plan to steal for the immediate satisfaction of their hunger. Notwithstanding, stealing with the intent to sell “bitter” cassava is not a lucrative business in the area because there is yet to be a market for processed products from “bitter” cassava. Since processing is a feminine activity thieving of “bitter” cassava is not lucrative for young males that have no inclination for processing.

The weight with which the bitterness and toxicity of “bitter” cassava confers protection for the vulnerable, especially impoverished households, was surprising but perhaps not unexpected. Vulnerability is synonymous with being female and poor(58, 202). Paradoxically the very persons that may be most at risk of exposure to dietary cyanogen are also the ones that mostly need the protection of the “bitter” and toxic cassava cultivars.

The fact that the cassava cultivars that they grew were “bitter” and yielded roots that required processing was not perceived as implying more work since the preferred staple food, *kondowole*, had to be made from soaked and fermented roots. The effectiveness of the processing method in removing cyanogens was evident by the low levels of urinary thiocyanate. Only in times of dire need was the processing of the cassava roots adjusted to provide a meal to tie them over until completely processed roots were available.

It is clear that the categorisation of cassava cultivars into “cool”, alias “sweet”, and “bitter” is based on the overall taste and the risk for poisoning if eaten raw. The classification into “cool” and “bitter” implies that cassava constitute two food crops with two well-defined functions in the food system.

## VALIDITY OF FINDINGS

The main investigator conducted the fieldwork with the same team of two field assistants from the very first study in 1994 to the last study in 1997. This meant that the farmers became familiar with the team and the rapport established by returning yearly improved validity of the findings. It was crucial for the outcome that the investigator and the assistants were fluent in Tumbuka, the lingua franca of the northern Malawi. The main investigator and one of the field assistants had a fair knowledge of Tonga while the other field assistant was fluent in Tonga. The results of the qualitative interviews were later translated into written English by the main investigator making it possible to include the other co-authors in joint interpretation based on their fields of expertise.

All parts of Nkhata-Bay district had similar demographic and socio-economic composition and were accessible by road during the dry season. However, the agricultural extension planning area of Chikwina, the one excluded in Paper I, had a road that was rather difficult to navigate and was subsequently excluded from the study. The cultivars identified in Paper I were commonly grown throughout the district although the proportions differed according to the local area preferences. The consistency of the findings in 13 different sites strongly supports the validity.

The elucidation of the reasons for growing “bitter” cassava comprised a sequence of research methods commencing with qualitative and semi-structured interviews, followed by the use of bio-chemical analysis, a sensory panel and ending with DNA finger printing. The consistency of the findings using widely different methods confers considerable validity to the findings. The qualitative interview in the first study was done with the staff from the research and extension services as well as farmers from the communities and included both sexes and age groups. However, the interviews focussed on active female farmers as they were the ones that were largely responsible for the farming and processing of cassava in the studied communities. The qualitative interviews with the female farmers were done with colleagues with different professional background and conduct. This caused a bias in some of the responses from the respondents as was evident by listening to the recordings. However, by triangulating interviews in different settings, with different compositions and doing several one to one interviews corroborated the main findings. The use of qualitative interview methods was important in the elucidation of the sensitive issue of thievery, especially for female-headed households.

A number of the focus group discussions and interviews were audio-recorded and video-recorded and some were not. At times the use of such sophisticated equipment caused an unusual interest in the studies and the interviews became longer than usual. However, ample time was taken in each community to establish rapport between the investigators and the farmers.

The selection of the two communities was done purposively for Studies II, III, and IV. Given the same findings in all areas included in Study I and the similar socio-economic status in the whole district these two communities can be regarded as being representative of the district. Selection of a lakeshore community was based on households having access to water throughout the year for the soaking of cassava, an all weather-road and relative proximity to the research station for transport of samples within one hour's drive for further analysis. The household census provided the basis for the inclusion of all households in a well-defined geographic area in the quantitative studies that had a very low level of non-participating subjects. Since the woman was the main respondent in the study, the presence of the woman was deemed critical in providing the information on cultivation, cultivars grown, processing and consumption. Only two farmers did not participate in the study because their husbands did not allow them to.

Some recall bias was inevitable in Study II since retrospective food frequency interviews depend entirely on recall(183). Notwithstanding, cassava comprised the daily meal of almost everyone in the whole community. The estimate of dietary cyanogen exposure was related to cassava consumption during the past seven days and it was unlikely that forgetting to mention other foods would have caused much bias.

The participants included in Study II provided a urine sample that enabled the comparison of cassava consumption with dietary cyanogen intake as estimated by urinary thiocyanate and linamarin levels. Since the half-life of thiocyanate is up to 7 days the use of the food frequency questionnaire for the past seven days implies that this measures the same period as does the biomarker for dietary cyanogen exposure. On the other hand the half-life of linamarin is only 24 hours. Given the tendency of respondents to forget reporting the consumption of snacks and other in-between meals it is not surprising that the levels of linamarin do not reflect the reported cassava intake during the last seven days. Furthermore, linamarin excretion could only come from the consumption of cassava whereas the metabolite thiocyanate could have come from other sources such as cyanide from firewood smoke. This lack of specificity of thiocyanate can explain the high values for those that did not consume cassava during the past week.

The 28 households in study III were consecutively selected on the basis of growing one or more of the ten most common cultivars in terms of the proportion growing it within the two communities. The consecutive selection was logistically necessary and there is little reason to believe that it created a selection bias.

Sensory analysis is a subjective perception of bitterness and sweetness. However, we applied robust standard methods for sensory panels and achieved a high degree of precision in double blind tests ( $r = 0.95$ ). Using a mean taste score of 12 independent panellists also made the final taste score more objective and specific. Chemical analysis was controlled for quality with double determinations and the repetition of the estimates yielded a correlation of  $r = 0.99$ . The main variation in the determination of cyanogenic glucoside is caused by the homogenisation step. Study III used a new and validated homogenisation method and was operated by the inventor(195).

The design of study IV entailed asking farmers to identify plants belonging to a specific cultivar from their own fields. The other option would have been to randomly select a plant and ask the farmers to identify which cultivar the plant belonged to. The study design chosen corresponded to the aim of the study which was to assess how well farmers can identify plants as belonging to a particular cultivar. It should be noted, however, that the study design is not able to determine the proportion of plants that farmers are able to identify.

The use of a botanical descriptor focusing on colours and shapes of major morphological characteristics depends on subjective judgements. Undoubtedly, the botanical descriptor could have been improved for the local scenario, however, it was deemed important to test the performance of this broadly used descriptor. Most of the polymorphic short sequence repeats (SSR) loci used to study the genotype composition of 232 plants were highly polymorphic and more than one allele was common in each locus. A complementary study in 1997 on additional cultivars showed that the eight SSR loci were sufficient to separate most of the genotypes grown in the area.

## **FARMERS' KNOWLEDGE OF TOXICITY**

In the qualitative interviews farmers stated that they equated the degree of bitterness in raw cassava roots with the risk for poisoning. This finding was verified in structured interviews by the finding of a strong correlation between the farmers' scoring of bitterness and their scoring of the risk for poisoning for each of the cassava cultivars grown. Their perception of bitter taste being equated with the risk for poisoning was confirmed by the strong correlation between the independent taste assessments of a trained sensory panel and the chemical determination of cyanogenic glucoside levels.

It is evident that people that use "bitter" cassava cultivars as their staple food have the knowledge about its potential toxicity(43, 140). Already in 1924 the Swedish ethnographer Nordenskiöld wrote in his ethnography of the Mojos in Bolivia;

*“There is nothing remarkable in the employment of a poisonous plant for the sake of the poison, but it is extremely remarkable that the poison should be extracted from a plant in order that the plant may be used as food, especially in the case of bitter manioc. There is the possibility that it was the poison they wanted.... By some chance they discovered that the pulp left behind could be eaten without ill-effect”(4).*

Other authors have suggested that the toxin in cassava was perhaps discovered by accident when the cassava juice was used as a fish poison(7). Outsiders have learnt by experience that “bitter” cassava is poisonous. The European colonial occupation of South America resulted in several early written reports on the toxicity of “bitter” cassava roots. Fermin as reported by Dunstan(45) reported that from 23 kg of cassava roots he obtained 87 g of an intense poisonous distillate. This publication may be the first written report of cassava juice being used to execute humans. Apparently, it was common practice for European slave owners to administer 35 drops of cassava distillate to a condemned slave. Within a few seconds the slave was reported to be poisoned to death(45). Evidently, the knowledge of the potential fatal effects of “bitter” cassava roots seem to be well understood by those that know how to process it before consumption as a staple food as by those that find it pointless to steal poisonous roots that cannot be eaten directly. Unless the thief is willing and able to invest labour in processing and to wait for the days necessary for processing the returns of stealing “bitter” cassava are virtually non-existent.

## **A PREFERENCE FOR “BITTER” CASSAVA**

The present studies show that the farmers divided cultivars into either “bitter” cultivars or “sweet” cultivars that are referred to as “cool” in Northern Malawi. A key finding of the first qualitative interview studies was that all the farmers had a strong preference for “bitter” cassava cultivars as a staple food. These findings concur with findings from South America(96, 98, 99, 104). Similar findings have also been noted in Africa(14, 40, 52, 106, 110, 114, 119). The most important reasons stated for the preference of “bitter” cassava in both the qualitative and quantitative interviews were the protective effects that the poison in cassava confers on the plant. These preferences can be assumed to be similar to those documented from other areas of Africa(6, 52, 57). The multiple reasons that were given for “preferring” bitter cultivars are discussed under seven themes; food security, theft, and animal destruction, storage of processed products, taste of end-products, high yield, and women’s status.

### ***Food security***

Although often vaguely understood, the concept of food security was well established in the studied community. It had a specific word *kuvikilia* in Tonga, the local lingua franca of the district. The main preference for growing “bitter” cassava for the farmers was linked to food security. The fact that roots from “bitter” cassava cultivars contain high levels of cyanogenic glucosides demands that processing be done to be rendered safe for consumption. This results in a pre-requisite for time and energy expenditure planning by the households. There was a tarmac road linking Nkhata-Bay to Mzuzu, the largest town in Northern Malawi and to major urban areas in the southern part of the country. In spite of this there was hardly any market demand for raw or processed “bitter” cassava at the time of the studies. This was partly due to the historical political view of cassava in Malawi. The late president Hastings Kamuzu Banda denounced cassava as an inferior food in comparison to maize throughout his political era. Notwithstanding, as of this year this is no longer the case. The research assistant Sidney Simkoko that was involved in these studies wrote to inform at the time of sending this thesis for publication. *“You would be surprised my friend to find kondowole on the shelves of the biggest supermarket chains in Mzuzu”*. Why, because the maize crop, as always feared by the farmers in Nkhata-Bay, failed in 2001 and there is now an acute shortage of food throughout Malawi. Within a few years urban populations will probably resort to eating products made from “bitter” cassava (<http://allafrica.com/stories/200110050269.html>).

These studies show that cassava is grown in Nkhata-Bay throughout the year and roots are kept in the ground as a living granary. For these farmers the alternative of not growing “bitter” cassava is to expose the household to imminent hunger, and this option is merely out of the question. Although “cool” cassava cultivars were also grown, they were planted on such a minor scale that they did not attain the same importance for food security. The raw roots from “cool” cultivars had a market. However, farmers did not trust themselves to grow too much “cool” cassava because either they would be stolen or if they escaped from thievery then the farmer would be tempted to sell the “cool” roots and their food security for the family would be compromised.

The need to temporarily abandon the fields of a farming household has been described as a reason for preferring “bitter” cassava. Studies in Asia and Africa during the colonial period(40, 52, 102, 106, 107, 110) showed that farmers regarded “bitter” cassava as a food security measure against unforeseen events such as famine, war or raids by other war-faring groups. It was possible to abandon one’s home and to leave behind the “bitter” cassava and come back two or three years later and still find the crop in the fields. Due to the bitterness and poison in cassava it was neither eaten by wild animals or humans that lacked the knowledge of or time for processing.

The processing of cassava into flour has been shown to occupy approximately 26% of the total daily time budget and 45% of the total energy expenditure in Amazonia(145, 146). It has also been shown that processing is not so much to detoxify as it is to acquire the desired end-products(6, 52, 97 114). However the very protective measure that the glucosides bestow can also be the very cause of transitory hunger or dietary cyanogen exposure due to the very requirement of several days of processing. Studies by Dufour in Amazonia have shown similar findings among the Tukanoan Indians where a minimum of seven days is required to produce a safe product with the desired flavour and texture(98). Likewise, the present studies also found that a household could be food insecure for several days while at the same time having plenty of cassava in the fields. This will occur if the regular course of harvesting and processing is interrupted due to an unpredicted disease, death, or other disasters that prevent the woman from processing as is routinely done.

Mechanical mills for grinding the dried roots into flour were time and again reiterated as the most urgent need for the women. The drudgery of manually pounding soaked and well dried roots each day into enough flour for the making of the staple dish *kondowole* was a much bigger problem than the other tasks related to processing. Many women stated that considerable time could be “released” for other chores if mechanical mills were made available to them. If they only produced “cool” cassava cultivars, their preference for the desired end products would still dictate that they process the roots in order to get the flour as is customary done with “bitter” cassava. Neither would the pounding of the roots differ. Therefore, an introduction of “cool” or no cyanogenic glucoside cassava cultivars would not cut down on their work. It could be concluded that the farmers’ preference for “bitter” cassava cultivars does not add any extra work for these women. Microeconomic studies show that technological improvements and market access can be particularly beneficial for female and household welfare if focused on cassava farming systems(203). The women should be relieved from the drudgery of pounding cassava roots into flour with stone-age equipment such as grinding stones and wooden mortar before other minor improvements in processing are addressed. Women perceived a reduction in their workload as something that could indirectly improve the food security of the household since there would be more free time for other activities.

### *Theft*

It became clear from the first qualitative interview studies that almost all the farmers mainly preferred “bitter” cultivars because they deterred human theft. It was perceived inconceivable to only plant the “cool” cassava cultivars because that would have implied a very high risk for thievery and inevitable hunger in the family. The structured interviews with a sample of 176 representative female farmers revealed that 90% considered it very important to grow “bitter” cassava cultivars because they conferred protection against theft.

The protection against theft was most probably of special importance to the communities that lived in the densely populated lakeshore zone. The perpetrators were often presumed to be young men that are in need of quick ready to sell or eat cassava. It was obvious to all farmers why bitterness and toxicity of roots conferred protection on the plant. In this district, market sales of cassava are almost always restricted to the “cool”/”sweet” cassava. Therefore, to steal “bitter” cassava for the purpose of selling would be futile. Since women do all processing the young men that often do the stealing do not indulge in processing of the roots. The probable reason being that a man that would be seen processing cassava would create much attention for himself. Ironically, the poison in cassava is therefore proven to be a deterrent for thieves and a protective mechanism for the female farmers.

The late Nemobozanga Sauti is renowned for his research on cassava in Malawi. Already in 1992 he reported that theft by humans was a major constraint for cassava production in Malawi. He had shown that “sweet” cassava was more often stolen than “bitter”(119). Several studies in South America(4, 7, 97), Asia(46, 100) and in Africa(6, 14, 52, 60, 118, 120) have shown that lowered susceptibility to theft is the one characteristic that is strongly associated with a strong partial preference for “bitter” and toxic cassava cultivars. As found in Malawi, “sweet” cassava is as in other parts of Africa perceived as being inferior to “bitter” cassava. The “sweet” cultivars are compared to children that are soft and vulnerable(52).

Why has the issue of “bitter” cassava curbing theft not received its due attention despite being reported in early and contemporary literature as the main reason for growing “bitter” cassava. The reason is probably that thievery of food is almost always a very delicate issue to document and report. Perpetrators, victims and local community leaders are all hesitant to share information about theft with outsiders. Thievery of food crops is equally sensitive and politically volatile to discuss and report openly by national authorities and researchers. Despite the issue of theft being taboo, it is not uncommon to find whole communities identifying pilfering of other peoples’ food crops as a coping mechanism in response to food shortages(204). The finding that “bitter” cassava curbs theft is no exception to these studies, but the rate at which it is cited as the main reason is cause for concern and more symptomatic of a larger problem within the agricultural, food, health, economic and development chain in Malawi. Thievery may not be stipulated as a reason to grow bitter cassava in other parts of Africa(53), where “bitter” cultivars may be preferred because of the characteristics that the end-products have(155).

Theft from cassava demonstration fields on research stations has often occurred in agricultural research trials(113). However, these important findings are rarely published because the observation of theft is perceived as a failed field trial. Theft of plants from trials are important research findings and it is important to document and publish that certain cultivars are more prone to theft.

### *Animal destruction*

In the quantitative interviews 74% of farmers stated that curbing of animal destruction was a major reason for growing “bitter” cassava cultivars. The qualitative interviews clearly indicated that farmers in the sparsely populated mountainous parts of Nkhata Bay district had even more difficulties with crop destruction by wild animals, such as wild pigs, monkeys, moles and other rodents. Interviews in this area revealed that “bitter” cassava cultivars were often the only crop that could be left in the fields with some degree of security. In isolated parts of the district, the numbers of the wild pigs and monkeys were so large that fields had to be watched and protected night and day. In these areas “cool” cassava was not an option, except very near the homestead as snack provisions.

Though predation by herbivores is often cited as a reason for growing “bitter” cassava (14) there are doubts about the importance of this concern by farmers due to lack of quantification of such destruction(99). In northern Malawi larger animals seem to decline in numbers but the smaller animals like monkeys and wild pigs are acculturated to live close to humans. The lack of quantitative data on the destruction caused can not be seen as an argument against not acting upon the farmers’ strong desire

to have their fields protected. In the studied area farmers complained that the forestry rangers do not have the manpower and arms needed to keep the population of the wild monkeys and pigs under control. The farmers' only remaining option is to rely on "bitter" cassava cultivars.

Rodents can destroy cassava fields(120). In some areas farmers use cassava fields as hunting grounds for such small animals and they constitute an important protein source in the cassava diet(65, 67, 205, 206). "Sweet" cassava is planted as bait for animals and snares are laid out to trap them. This is common practice in the Amazonia where Tukanoan women plant "sweet" cassava to attract a small rodent, *Dasyprocta punctata*. It would appear that the herbivore attacks are partly due to deforestation and population increases. However, slash and burn agriculture in forest areas always attracts wild game animals such as, peccary (wild pig, swine), rodents, agoutis (guinea pig), monkeys and other vermin(65, 205, 206). The bitterness and toxicity in cassava roots may thus have been of importance for protection of fields since the time of domesticating cassava in Amazonia.

### *Storage of processed products*

Farmers regard the products made from roots of "bitter" cassava cultivars as storing better than those made from roots of "sweet" cassava cultivars. These findings are also supported by similar findings in Zaire(52) where farmers reported that soaked and dried roots, so called "cossettes", made from "bitter" cassava keep better and that dried cassava chips keep longer and are less infested with insects and weevils than chips made with "sweet" cassava(115). It may be assumed that the difference in storing is due to the cyanogenic substances *per se*. However, since these are reduced to negligible levels during processing it seems more probable that the difference is due to other genetic factors. There is a clear difference in the general use of roots from the "bitter" and the "cool" cultivars to the extent that they may be perceived as constituting to different crops in the farming and food system. "Sweet" cultivars are grown to be eaten as snacks or to be directly prepared without processing, whereas the "bitter" cultivars for centuries or more have been used for processing into storable products to be eaten as the staple food. This could explain the existence of a genetic coupling in "bitter" cultivars between good storing qualities and high cyanogenic glucoside levels. The reasons for growing "cool" cultivars is to eat roots directly fresh as snacks or thirst quenchers, and their characteristics have therefore not been coupled to good storing characteristics.

### *Taste of end-products*

Farmers claimed that products made from roots and leaves of "bitter" cassava cultivars had a superior taste and appearance compared to when the same products were made from "cool" cassava cultivars. The staple food *kondowole* was perceived as being easier to mould and to roll into balls, and the cassava leaf relish was more tender when made from "bitter" cassava cultivars. Several studies show that "bitter" cassava roots have better starch quality in the production of cassava flour and other end products (11, 52, 97, 114). Studies by Dufour(97) show that *manicuera*, a beverage made from the washing water from processing of roots from "bitter" cassava cultivars have a slightly sweeter taste and *farina* flour a slightly more sour taste when made from "bitter" cassava than when made from "sweet" cassava cultivars. It is argued that cassava processing is foremost not done to detoxify roots but to attain the desired food products, such as the flour for making *kondowole*(96, 104, 155). The end-product quality is therefore essential for the selection of which cultivars to grow.

It may be speculated that the cyanide released during processing may have an impact on the starch and thereby affect end product quality. However, it seems again that over centuries the "bitter" cultivars have been selected to have high dry matter content and this has resulted in higher quality of the finished products made from these cultivars. In the area of these studies the preferred method for processing cassava is soaking followed by fermentation and the "bitter" cultivars used probably have genetically coupled characteristics that make produce end products with the desired qualities. Another contributing factor may be that since the poison acts as a deterrent for animals and insects

the plants of “bitter” cassava cultivars are stressed to a lesser extent than are the plants of “cool” cultivars.

The preference stated for leaves from the “bitter” cassava cultivars is similar to findings in other studies(70). It is not understood why cassava leaves of “bitter” cultivars may yield a more tender relish nor why mosaic virus infested leaves are more preferred than non infested leaves. In fact there are very few studies that have looked at the taste properties of tropical plants that are consumed locally(76, 129, 155, 207, 208).

### *High yield*

Yield was a reason for growing “bitter” cassava that emerged in the first qualitative study as well as in the structured interviews where 90% of interviewed farmers stated that high yield was a very important reason for growing “bitter” cultivars. For the farmers the concept of yield meant the number of roots, their size and the weight of the roots as a function of the overall environmental conditions that the cassava plant had grown in. Since the roots for making the staple food had to be peeled and soaked and fermented, they were preferred to be “sizeable” for easy holding in the hand and for peeling. These preferences may have affected farmers yield concept.

There are some few studies showing weak correlation between bitterness of cassava cultivars and higher yield(97, 124, 137). It can be hypothesised that cyanogenesis biologically improves yields by protection against microbes and insects. However, McKey and Beckerman(99) proposed that the seeming contradiction in the literature regarding the relation between bitterness and glucoside levels on one hand and higher yield on the other hand is due to yield being viewed as an intrinsic biological factor of the plant. Yield should instead be a product of interaction between the plant, environment and man-made interference, i.e. the sum of the production of that cultivar that is finally obtained by the farming household. In using this yield concept farmers can be correct in implying that yield is causally related to toxicity, via the protective effects of toxicity against herbivores and theft(116, 117, 209). It could also be that the “bitter” cultivars have been systematically selected for high yields since they provide the staple food, whereas the “sweet” cultivars have been less important to select for yield because they are only eaten as snacks and pot-vegetables.

When experimental trials have been conducted at agricultural research stations, they have failed to find any association between yield and the cyanogenic glucoside levels of cassava varieties(113). Since trials at the agricultural stations are performed differently from farmer’s way of relating yield to the toxin in cassava, it is no wonder that there is this discrepancy. For the farmers, cassava farming is part of life in her/his community and theft reduces yield, whereas the researcher will regard theft as an unsuccessful field trial from which no conclusions are drawn.

### *Food security and women’s status*

To be a single woman in rural Malawi means that you are more vulnerable than if you are a wife to “someone”. A single woman may be respected if she has a “good social standing”, is a sister or a daughter to some respectable male member of the society. The first qualitative interview studies and later interactions with impoverished single women revealed that for a single woman without “good social standing” growing “bitter” cassava ensured that she would have food for her household. Destitute single women stated that if they planted “cool” cassava in their fields, especially at some distance away from the homestead, the likelihood that they would be the one to harvest was virtually nil. Theft of crops was not only limited to single women, but they were much more likely to suffer from theft. An “insignificant” single woman that dares to stand up for herself and dares to complain about theft would inevitably suffer from name-calling. Such verbal abuse would often originate from the very same young men that did the stealing.

A woman's position in society can be understood by her direct or indirect access to critical resources, in this case food. In many contemporary rural communities in Africa the important thing is not who owns something, but rather who can control it and thereby use it(210). Although control is an important aspect of production it does not automatically follow that the one contributing with time and labour also controls the products generated. Gender relations within a society have significant impact on a woman's capacity to control various aspects of the social relations of production(211).

Studies in South America have shown that de jure or de facto single women, conduct agriculture differently especially in subsistence agriculture(212). They tend to have smaller fields that are cultivated intensively over longer periods, have more diverse weed compositions, plant less labour intensive crops and use creative subsistence strategies such as child labour, barter, off-farm labour and cash to effectively farm. The situation was quite similar in rural Malawi. Women emphasised the need to plant "bitter" cultivars to protect their fields from theft. But in addition they also needed to establish contacts with at least one powerful male member with good social standing in the community that could be counted upon for added protection of various forms. Since most thieves were from within the local community they would know to avoid the fields of women having contacts with influential males. Therefore, liaisons with powerful men might provide partial immunity from pilfering of crops and improves the overall welfare of vulnerable single women.

The situation of single women in Africa is very precarious(213). Legally women have severely circumscribed rights and in the gender system men control their lives and that of their children, but males are often not obliged to be responsible for them(211). Most women have to assert that they have behaved appropriately in order to enforce their claims within society. That is as wives, virginal daughters, good mothers or virtuous widows. Alternatively they could achieve powerful status and protection by providing sexual favours and by having an acknowledged relationship with an influential male member of the local society. However, then they are often condemned to be called "loose" women by both men and "virtuous" women(211). Therefore, it comes as no surprise that such women find refuge in everything that can offer some extra degree of protection in their lives. In communicating their harsh reality it constitutes a challenge to be comprehensible to all without being impertinent.

## DIETARY CYANOGEN EXPOSURE

Low mean urinary thiocyanate (SCN) was found among the female farmers that frequently consumed foods made from cassava roots containing initial high levels of cyanogenic glucosides. The daily *kondowole* consumers had a mean urinary thiocyanate of 57  $\mu\text{mol/l}$  that was slightly higher than for the non-consumers. However, the mean SCN level for all the women studied corresponded to that found of Swedish subjects  $50 \pm 4 \mu\text{mol/l}$  and that were non-habitual cassava consumers. To estimate cassava intake, a food frequency questionnaire was used to assess the average diet during the past seven days. Food frequency questionnaires provide a good proxy for the frequency of food intake(183, 186, 214) especially for foods eaten frequently and in large amounts. Since we did not use portion sizes or recorded mean intakes, we therefore, compared the dietary cyanide exposure estimates using urinary thiocyanate as a biomarker (215). As the half life of thiocyanate is approximately one week it was possible to compare last week's consumption with observed urinary levels. A modest but statistically significant difference in mean urinary SCN between women with different kondowole consumption frequencies indicated that a slight dietary cyanide exposure does occur in the studied area.

One concern that has shadowed cassava diets is the supply of sulphur amino-acids that are low in supply in the roots and that are needed for detoxifying dietary cyanide to thiocyanate(22, 216). This area was situated along the lakeshore and almost all the families reported having eaten fish at least once a week. This provided the population with a regular protein intake rich in sulphur amino acids providing the substrate for thiocyanate formation. There is thus no reason to believe that their thiocyanate levels do not reflect their dietary cyanide intake.

The mean urinary SCN levels were only one tenth of the levels found in consumers in konzo stricken areas(167). The populations affected by the paralytic disease konzo used the same processing method but due to extreme poverty they had shortened the processing in times of food shortage. The findings in Malawi show that these women had minor dietary cyanide exposure. This would indicate that the dietary cyanogens were effectively removed during processing. Their mean linamarin levels were also low indicating that all types of cyanogens had been removed effectively. The processing method used in the area, soaking followed by sun drying is known to be the most effective method of processing if conducted correctly(89, 149). Studies in Amazonia have shown that even roots from the most toxic cultivars can be effectively processed and the cyanogen content reduced to negligible levels simply by processing(143). The chemical urinary analysis thus confirms women's perceptions that the potential toxicity of cassava does not constitute a health problem in this community. It is interesting to note that the studied area at the time of the study lacked market opportunities for bitter cassava roots as well as flour made from them. Opportunities appear to be developing in Malawi and that could potentially change traditional processing and perhaps even induce short cuts in processing. This was the case in Congo when impoverished rural populations started to sell processed roots of "bitter" cassava without having access to milling opportunities for their own flour(167). The probability of similar events occurring in Malawi is there, however, Malawi has a smaller urban population whose purchasing power may not be so strong. This would limit the expansion of urban cassava markets while ironically retaining the safe processing methods.

Of the 23 cases that had SCN levels above 100  $\mu\text{mol/l}$  only three women admitted to shortening the fermentation period due to food shortage attributed to a lack of time planning. This suggests that dietary cyanide exposure could increase if some social changes would make short-cuts in processing more frequent. The justification for these incidents was often attributed to the laborious drudgery of pounding. Farmers usually just harvested what they could pound without wasting any food. The longer the dried roots were stored the harder they were to pound. Hence, there is always the possible risk of running out of food.

At least 55% of the farmers interviewed in the quantitative study consumed cassava in the form of the staple food *kondowole* on a daily basis. The consumption was not as frequent as in the normal years due to the area having experienced excessive rains and there being a shortage of mature cassava. Households that consumed cassava frequently had a higher mean age and were often female-headed households.

When processing cassava roots by soaking and fermenting the farmers did not make a distinction between the roots from "bitter" and "cool" cultivars. This distinction was only made when using the roots from the "cool" cultivars as a snack with no prior processing. The fact that urinary linamarin was low indicates that farmers know which roots to select to eat as raw snacks and having low glucoside levels.

The absolute requirement of processing to obtain safe food could actually predispose a family to food insecurity(22). This thesis argues two pre-requisites should be introduced simultaneously. These include mechanising processing at the village level(159, 217) and opportunities for markets and networking(218, 219) created for the outlet of some these products. This would ensure reduced likelihood of increased incidents of poisoning, and possible short-cuts in processing. The new found time and income gained could possibly be used to diversify the diet. The three women that stated that they had experienced acute poisoning clearly stated that it was the pounding that really made them "careless". It was regarded as an embarrassment to have to go and ask a neighbour for some well-processed flour because then a woman would be perceived as lazy. To overcome this a woman took the risk of preparing food from improperly processed cassava. There is evidence that when incomes and population increase the demand for both raw cassava and processed cassava products increases(113, 218). When promoting marketing of cassava from areas where it has been a subsistence crop and that has been processed with traditional skills and tools attention should be paid to the effectiveness of proces-

sing(152). It would be naïve to believe that modifications in processing would not be made when processed cassava becomes a commercial product.

## **BITTER TASTE AND CYANOGENIC GLUCOSIDES IN CASSAVA ROOTS**

The strong correlation ( $r > 0.8$ ) between cyanogenic glucoside levels and mean bitterness score obtained from the sensory panel for the 492 roots is consistent with farmers' unanimous statements that taste predicts toxicity. When the taste scoring of each individual cultivar is desegregated there still remains a statistically significant association between bitter taste and cyanogenic glucoside. There is a wide dispersion of glucoside levels among the high scores of bitter taste. This may be explained in two ways. First the taste score may have been too narrow or secondly the taste buds may have been saturated. In spite of this effect at the upper end there still was a strong correlation between taste and the level of cyanogenic glucosides. This is consistent with linamarin itself being the conveyor of the bitter taste. However, as the tasting was done on intact root pieces the strong correlation in itself does not prove that it was the cyanogenic glucoside molecules that elicited the bitter taste. It is theoretically possible that the bitterness could be due to another compound, the levels of which must be genetically very tightly coupled with the glucoside levels. As shown in figure 3 there was only one root that had deviating values for the relation between taste and glucoside. This cannot be explained by a different genetic coupling of that plant since the other root of the same plant showed the same relationship between glucoside levels and taste, as did the other 490 roots.

Some earlier studies failed to find a correlation between cyanogenic glucoside levels and bitter taste of cassava roots (128, 131, 132) and thus conclude that taste cannot be used as an assay for determining toxicity. Yet other studies found a correlation between bitter taste and level of cyanogenic glucosides, and conclude that linamarin might be bitter, but that other substances might partly mask or alter the bitter taste(93, 133-135). The difficulty in interpreting some of these studies is due to the lack of information on the sampling of the roots and the detailed procedures used for tasting. It is worth noting that the early studies acknowledged that a remaining bitter taste in boiled or cooked cassava was an indication of not being safe enough to eat(79).

There is only one study(129) published on the taste of pure linamarin. The molecule itself was found to have a bitter taste. The study was conducted to clarify what caused the bitterness of cassava roots. In the peel of the roots a new compound, other than linamarin, substantially contributed to the bitter taste. However, in the parenchyma, i.e. the edible part of the roots, the authors did not find any other compounds that contributed to or modified the bitter taste of linamarin. The studies of this thesis support the statement by King and Bradbury(129) that cyanogenic glucosides, mainly linamarin, are the sole contributors of bitter taste in cassava roots. Interestingly the first researcher to extract linamarin stated already in 1906 that "*it had bitter taste*"(45).

The findings from the experimental study provide a rationale for the farmers practice to taste the tip of the root to ascertain risk for danger and for their perception to equate bitter taste with potential toxicity. A strong positive correlation ( $r = 0.65$ ) was also found between the glucoside levels and the taste scores given by the farmers when tasting the tip of the root following the up-rooting of the plant. Two factors can explain why the correlation was not as strong as when glucoside levels were compared to the outcome of the tasting by the sensory panel. Firstly that only one individual farmer tasted each root whereas the outcome of the taste panel was a mean score of 12 panellists tasting of the root. Secondly the farmers tasted the tip of the root whereas the taste panellist tasted different parts of one longitudinal half and it is known that there is a longitudinal variation of glucoside levels.

The results also strongly support that farmers have the ability to predict potential toxicity in cassava roots by using their ethno-classification into "cool" and "bitter" cultivars, as well as by their general experiences with each of the most common name given cultivars grown in the area. Their prediction

improved further when farmers actually tasted the root. Amazonian farmers' view of bitterness in relation to toxic potential was studied by Dufour (50, 97). According to Dufour(50), the way the Tatuayo Indians divide the cultivars into "sweet" and "bitter" reflects the cyanogenic glucoside levels that is either less than 100 mg HCN equivalents per kg fresh weight in the "sweet" or greater in the "bitter", respectively. The Tukanoans also correctly associate the poisonous quality with bitterness of taste (97).

Interestingly 100 years ago it was shown that the mean HCN yield from roots of "sweet" cassava cultivars from Trinidad was 70 mg HCN equivalents per kg(36) and that 17 "sweet" varieties from Colombia had a mean of 17 mg HCN equivalents per kg(37). The commonly used chemical classification of "sweet" and "bitter", or innocuous and dangerous roots is based on the classification published in 1933 by Koch(100) and later quoted by Bolhuis(92). This has been the main reference for classifying "sweet" roots as having less than 50-100 mg HCN equivalents per kg.

In Malawi the roots from the corresponding "cool" cultivars had a mean of about 30 mg HCN equivalents per kg and these were regarded as being safe to eat raw. The taste panel distinguished some of these roots to be "very cool" and others to be "cool" and the mean glucoside level in these two groups was around 15 and 30 mg HCN equivalents per kg fresh weight, respectively. This would indicate that the threshold for tasting bitterness of linamarin when tasted in the matrix of raw roots is below 20 mg HCN equivalents per kg as graphically supported by figure 3. This is consistent with the finding that the taste threshold of linamarin in water solution was about 10 mg HCN equivalents per liter(129) since the matrix of the root would mask some of the taste. Interestingly the declared safe level of cyanogens in processed products as defined in Codex Alimentarius(220) is 10 mg HCN equivalents per kg dry weight. The level, 50 mg per kg fresh weight, defined as innocuous(100) is therefore 15 fold higher. The lowest taste detectable level of cyanogen glucosides in the present study corresponds to about 60 mg HCN qn per kg dry, i e. 6 fold higher than the codex level. This is inspite of these roots being eaten fresh for centivies without any appaent side effects. This may be explained in part by the fact that a large part of the linamarin in raw roots passes the body and is excreted unchanged in the urine(221). This means that the recommended Codex Alimentarius level includes a considerable safety margin.

## IDENTIFICATION OF CULTIVARS

The Malawian farmers grow mixed plants of both "cool" and "bitter" cultivars in the same field and the plants appear seemingly similar. Advances in molecular genetics made it possible to study how well these farmers grasped cassava genetics when dealing with cyanogenesis in their farming system. The ten most grown cassava cultivars that were studied had all been obtained through informal exchange of stem cuttings and none could be traced back to international or national scientific breeding. The results in table 7 confirmed that the local ethno-classification of cassava plants into a high number of cultivars with local names reflects the skillfulness of African cassava farmers in maintaining and recognizing many different local genotypes of cassava. Farmers in this part of Africa have cultivated cassava for no more than two centuries(6), but appear to be good at identifying local cassava genotypes as the Amerindians whom have grown cassava for thousands of years(29).

The percentage of plants with "typical" genotypes was especially high for the cultivars 'mbundumali', gomani and nyamakozo. Discriminate analysis of the morphological characters showed that plants with mbundumali genotype differed distinctly from that of plants with other typical genotypes. A high agreement could thus be expected between farmers' identification of the 'mbundumali' cultivar and the typical 'mbundumali' genotype. However, the high agreement for 'gomani' and 'nyamakozo' was surprising, since we found considerable morphological overlap between plants with either of these two genotypes as well as with plants with other genotypes. The reason for the high agreement cannot be attributed to the plants of these two genotypes being very common in the fields, since 'nyamakozo', in contrast to 'gomani', was the least grown cultivar (table 5). Neither can it be attributed

to the farmers having planted different cultivars in a recognisable pattern in the fields. We observed that farmers' identification of plants of each cultivar always involved thorough searching throughout each field with careful examination of a high number of plants.

The high agreement proves that farmers have extensive knowledge about detailed morphological characteristics that are distinctive for each genotype. However, it seems that the link between cultivar name and specific genotype may be a local phenomenon, since the cultivar 'depwete' was easily identified in one village and only 3 km away it was wrongly identified. Nevertheless the perfect identification of the cultivar 'gomani' and that near perfect one of 'mbundumali' (92%) would still speak for farmers striving to keep their cultivars as single genotypes. Since the name 'gomani' and 'mbundumali' are used for cultivars over a large part of Malawi it would be important to test if these names always refer to the same genotypes. The name may, within the country, refer to several genotypes of which only one is used in each area. The farmer's morphological skills in identifying the cultivars by name and correct genotype reflect a skill that is often overlooked. This would also indicate that farmers adopt cultivars in their farming system with certain recognisable distinctive morphological and in addition to this taste, yield and other characteristics.

Only 14 plants had a genotype "typical" of another cultivar. Nine of these had the "typical" genotype of 'depwete'. This cultivar had recently been adopted in the area, which may explain why farmers so frequently identified plants with this genotype as belonging to other cultivars. The "typical" genotypes of all ten cultivars were identical for all 29 farmers, but the "non-typical" genotypes of plants identified as a particular cultivar differed from one farmer to the next. This indicates that the aim of the farming community is to maintain cultivars with homogenous "typical" genotypes, rather than cultivars with a mixture of "typical" and "non-typical" genotypes.

Our findings also show that farmer's ethno-classification of cassava cultivars into "cool" and "bitter" predicts the cyanogenic glucoside levels of roots (table 7). It is remarkable that the farmers classified all plants with "typical" genotypes correctly with respect to their belonging to either "bitter" or "cool" cultivars. These farmers are adept at differentiating between genotypes having high or low levels of cyanogenic glucosides.

The necessity to differentiate between "cool" and "bitter" cultivars seems to have influenced the genetic structure of cassava. When the ten "typical" genotypes are plotted using the first two principal components of SSR alleles, the genotypes of the three "cool" cultivars were genetically separated from those of the seven "bitter" cultivars. This is the first molecular evidence supporting cassava farmers' statements that "cool" and "bitter" cultivars may constitute two different crops with different roles in farming and food systems. Amerindians also regard "bitter" and "cool" cultivars as two different crops however, agricultural science has yet to verify a genetic division using botanical taxonomy. Since active crossing of local cultivars could not be verified in our study area we find it improbable that the division of "cool" and "bitter" genotypes reflects a local selection. The division may have occurred in Africa or South America over centuries or the two groups of cultivars may reflect separate domestication thousands of years ago (99). When farmers talk of *killing* cultivars that do not act according to the classification of "sweet" (cool) and "bitter" it would indicate that this division is important to them and that it is maintained with reason and care.

In conclusion, biological knowledge of cassava cyanogenesis among small-scale African farmers enables them to simultaneously benefit from the protection it confers against theft and to effectively remove toxicity by processing. Ironically, toxic effects are rampant where protection against theft is most needed. These findings are of significance because development of non-cyanogenic glucoside cassava (85, 86, 222) does not appear to be the poor farmers priority. Such cassava(223) would be of minor significance for poor small-scale farmers whose livelihoods depend on protection of their cassava by the toxicity of the roots. Notwithstanding, cyanogenic glucoside free cassava may be useful where theft is rare and "cool" roots are consumed without processing, as well as to further the understanding of the biology of cyanogenesis.

Biotechnology undeniably has potential in the quest for food security in Africa (224, 225). In addition to marker assisted breeding, the advances in molecular genetics(226) also provide new tools for understanding the complex farming and food systems of poor small-scale farmers. The combination of molecular genetics with farmer participation (227, 228) provides a research tool that is a useful addition to the methodology needed to improve food security in sub-Saharan Africa.

The tradition of cassava processing lies predominantly in the hands of women(7, 62, 137, 146). Women have profound knowledge in processes regarding efficiency of processing plants(229). These studies show that women constantly experiment with different cultivars, to find the desirable traits based on overall performance under a variety of circumstances. As in other studies(230-232) the women become the main keepers of plant diversity whether knowingly or unknowingly. This cautions for more concerted women involvement in cassava breeding activities since the trend in agriculture in large parts of Africa is not only lending towards the feminisation (58) but also towards “tuberisation” of agriculture (56).

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# Paper I





# THE IMPORTANCE OF BEING BITTER—A QUALITATIVE STUDY ON CASSAVA CULTIVAR PREFERENCE IN MALAWI

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We conducted qualitative interviews in Nkhata-Bay district in Malawi to elucidate why farmers preferentially grow cassava cultivars providing bitter roots. Cassava was mainly grown to produce flour for making the staple food, *kondowole*. Plants were identified as belonging to one of the 54 mentioned cultivars with local names. All the farmers stated that bitter taste of roots predicted toxicity and necessity for processing. Cultivars were grouped into “cool” or “bitter” based on whether the roots could be eaten fresh, or required processing before consumption as *kondowole*.

Farmers strongly preferred cultivars grown for flour production to have bitter roots since this protected against theft, destruction by animals and deterred household members from unplanned harvest. Since processing is done

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by women bitterness empowers women to control the families staple crop. Bitter cultivars reportedly yielded more and roots produced an easier to mould *kondowole*. Bitterness and toxicity were not perceived as a problem.

**KEY WORDS:** Cassava, bitter, food security, folk taxonomy, cyanogenesis, cyanide, genetic diversity, gender, Malawi, Africa

## INTRODUCTION

The starchy roots of cassava (*Manihot esculenta* Crantz, Euphorbiaceae) are the staple food for over 400 million people, half of whom live in Sub-Saharan Africa. Cassava has gained this importance due to its ability to yield well, especially under adverse environmental conditions (Akoroda, 1995). The roots and leaves contain varying amounts of two cyanogenic glucosides, the most important being linamarin (Conn, 1994). Both genetic and environmental factors concurrently determine the glucoside levels in the roots (Bokanga *et al.*, 1994) and high glucoside levels are associated with bitterness (Sundaresan *et al.*, 1987). Raw roots from cultivars that provide non-bitter roots are eaten fresh or boiled, whereas the high levels of cyanogenic glucosides in roots from bitter cultivars must be reduced by processing. The term cultivar refers to plants of the same genetic variety that have been purposely selected for propagation through stem cuttings due to preferred agro-alimentary characteristics (Purseglove, 1968). Processing also improves shelf life, reduces bulk during transportation and improves palatability of the prepared dishes (Dufour, 1989; Nye, 1991). In Africa, labour intensive cassava processing is predominantly done by women (Hahn, 1989; Nweke, 1994).

A common method of processing bitter roots into flour involves fermentation by soaking (Coursey, 1973; Nweke, 1994), followed by drying and pounding. The flour is used to prepare the staple food, a dumpling-like thick porridge. If strictly adhered to, the soaking procedure is known to effectively reduce all cyanogen compounds to negligible levels (Tylleskär *et al.*, 1992). Disintegration of the plant cells by fermentation brings

the glucosides into contact with an endogenous glucosidase that breaks the glucosides down to cyanohydrins. These in turn decompose to the volatile hydrogen cyanide that dissolves in water or evaporates into the air during drying or cooking (Bokanga, 1995; de Bruijn, 1973). Interruption of the processing sequence due to food shortage may result in high amounts of residual cyanogens in consumed products and hence dietary cyanide exposure. Such exposure may cause acute poisonings, aggravate iodine deficiency and has been implicated as a causative factor in neurological disorders (Tylleskär *et al.*, 1992; Rosling, 1994).

In areas where cassava is the main staple crop farmers appear to preferentially grow cultivars that produce roots that are both bitter and toxic (Nweke and Bokanga, 1994; Purseglove, 1968). Paradoxically, even populations that have suffered acute toxic effects from cassava, seem to favour bitter and toxic cultivars, even though non-toxic cultivars are available (Essers *et al.*, 1992; Rosling, 1995). Therefore, they must have good reasons for preferring bitter cassava cultivars since their use obliges adherence to laborious processing methods to avoid acute poisoning (Coursey, 1973). Extensive knowledge of plant chemicals have been found in agricultural communities when carefully studied (Johns, 1990). Several studies have been conducted regarding preferences for cassava cultivar preferences as well as potatoes in South America (Boster, 1984; Brush *et al.*, 1981; Salick *et al.*, 1997). However, to our knowledge, the rationale for preferring bitter cassava cultivars among farmers in Africa has not been carefully studied (Bokanga, *et al.*, 1994), although this has profound implications for breeding and agricultural extension services (Richards, 1985).

We set out to elucidate perceptions about the potential toxicity of cassava roots and the possible rationale for preferring to grow cultivars with bitter and toxic roots among small-holder farmers in Nkhata-Bay district, the most cassava dominated area in Malawi. We used a combination of explorative survey methods with an emphasis on the predominantly women farmers (Morse and Field, 1995).

## STUDY AREA

Malawi has 10 million inhabitants of which 87% live in rural areas. Maize and cassava are the main staple crops. We studied Nkhata-Bay district (Figure 1), an area with 140 000 inhabitants (Malawi Government, 1991). Cassava has been the main staple food in this area for more than a century (Bontinck, 1974; Parke, 1891; Thompson, 1989). Nkhata-Bay is situated in the northern region along the shore of Lake Malawi and encompasses four agro-ecological zones: (1) the islands of Likoma and Chizumulo, (2) a lakeshore zone at 475–600 m above sea level, (3) an escarpment zone between 600 to 900 m above sea level and (4) a less densely populated mountainous plateau zone more than 900 m above sea level. The annual average rainfall is more than 2 000 mm, with a dry period from April to November, and the mean temperature ranges from 13°C in June to 35°C in November.

Nkhata-Bay mainland is mainly populated by the Tonga and Tumbuka ethnic groups whereas the population on the islands are mostly Chewas (Crosby, 1980; Agnew and Stubbs, 1972). All groups have a patrilineal kinship system and polygyny is commonly practised. Many families are female headed due to migration of the husband, widowhood or divorce. Cultivation is mainly done with a hoe and households have access to land through customary tenure. Cassava occupies more than 70% of the land cultivated by small-holder farmers (Msukwa and Pelletier, 1990; Paris, 1991). In addition to cassava, vegetables, rice and maize are also grown. On the islands and along the lake shore, fishing is a major livelihood activity. In the escarpment zone tobacco is grown and in the plateau zone tea, coffee, tobacco and pinewood are grown for cash income. Nkhata-Bay town, a semi-urban district administrative centre, is a major lakeshore port. There is an all-weather tarmac road contouring most of the lakeshore, whereas other roads are untarred.

For agricultural extension services Nkhata-Bay district was in 1994, divided into four Extension Planning Areas named Nkhata-Bay, Mpamba, Chintheche and Chitheka. The first three, situated along the lakeshore, were included in the study.

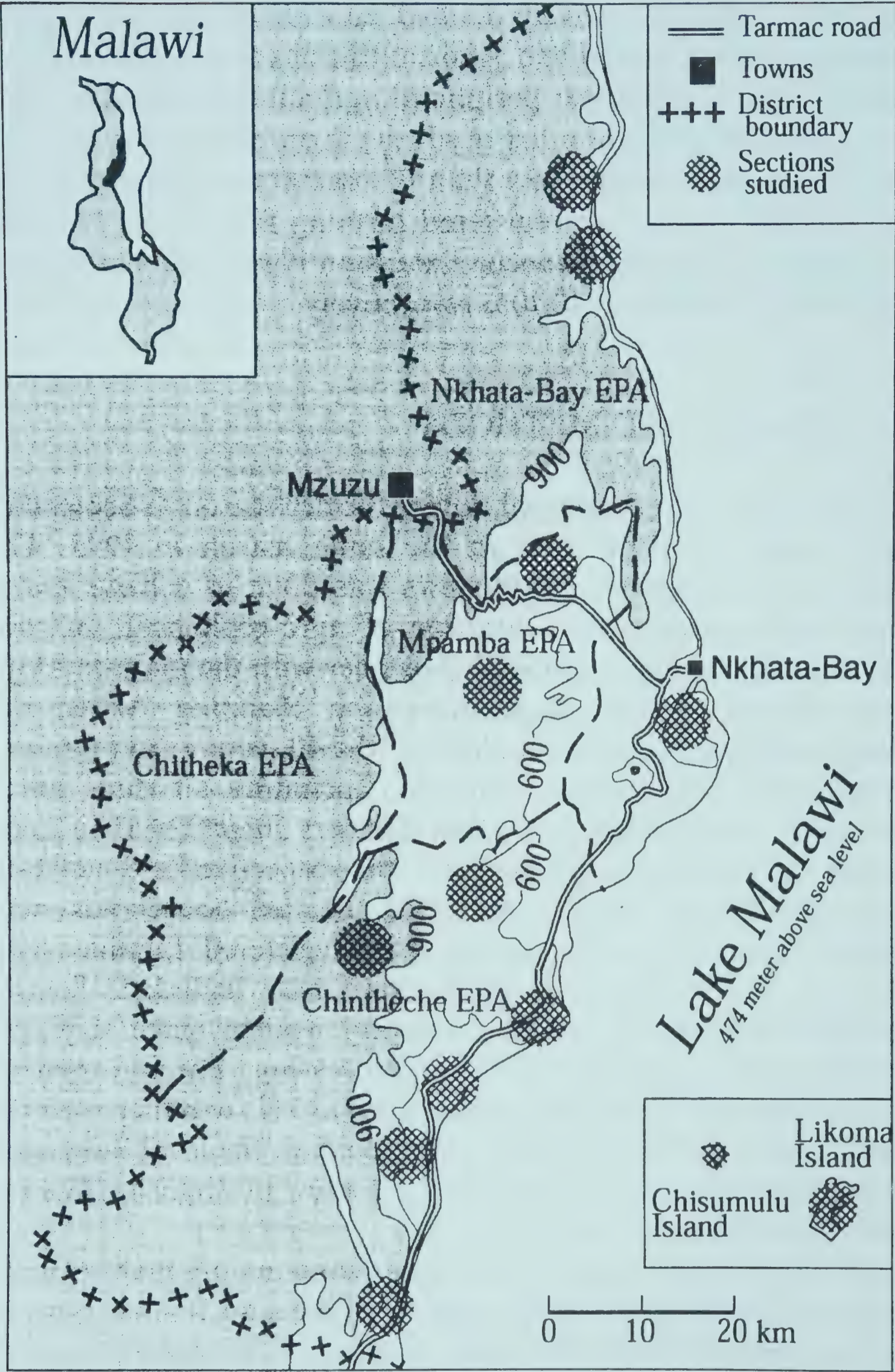


FIGURE 1 Map of Nkhata-Bay district indicating the location of the 13 sections studied.

They were administratively divided into 13, 13 and 14 sections, respectively, with one Field Assistant in charge of each section. Almost all of these most peripheral agricultural extension staff were males. They kept a list of all the farming families, defined as a household with no less than 0.2 hectares of farming land. In 1994 the number of registered farming families in Nkhata-Bay, Mpamba and Chintheche Extension Planning Areas (EPA) were 13 092, 8 301 and 10 705, respectively.

## MATERIALS AND METHODS

The fieldwork was conducted during the dry season in September and October in 1994, and in the same season in 1995. The selection of subjects for interviews was done to achieve maximum heterogeneity and variation of agro-ecological, cultural and socio-economic conditions. Together with the male development officers of the three Extension Planning Areas, the main investigator identified 13 out of the 40 sections to represent a maximum geographical variation including all three agro-ecological zones (Figure 1). The farmers interviewed in each section were selected together with the respective field assistant to represent both male and female headed households with different socio-economic status. Non-registered families with less than 0.2 hectares of land were also included. Special emphasis was placed on interviewing women since they are responsible for farming and food processing. The information was collected by the main investigator (LC-K) together with the field assistants. The interviews were done in the local languages Tonga, Tumbuka and Chichewa, and a few in English when the interviewees so preferred.

A total of 43 key-informants comprising mainly male agricultural extension staff, chiefs, notable male and female farmers and knowledgeable elderly women and men were the first source of primary data collection in each section. The informants were asked to recount their experiences and a checklist was used to guide the narratives. Participant observations, 12 in-depth interviews and 10 focus group discussions were thereafter interactively

used to elucidate the range of knowledge, practices, attitudes and beliefs related to cassava cultivation, processing, consumption and preferences for cassava cultivars (Morse and Field, 1995; Dawson, Manderson, and Tallo, 1993; Kreuger, 1994).

Field notes from all discussions, interviews and observations (Morse and Field, 1995) as well as 12 audio-recorded and six video-recorded interviews were transcribed into English and classified into salient themes according to content. Thereafter, analysis was done incorporating the multi-disciplinary background of the authors and the socio-cultural knowledge of the three Malawian authors. Noteworthy excerpts that illustrate the salient themes from the interviews have been quoted in English.

## RESULTS

To a great extent all three ethnic groups, Tonga, Tumbuka and Chewa live interspersed in the same communities. Cassava is the main staple food for all the ethnic groups in the 13 sections. Since there were no major differences regarding preferences for cassava cultivars or in the processing methods the results are presented jointly.

### *Cassava in the Farming and Food System*

Cassava has been the main staple food in the area for as long as the farmers could remember. For the Tonga ethnic group, cassava is the preferred staple food even when other staple foods are available. The other two ethnic groups seemed to eat as much cassava as the Tonga group but many of them expressed a somewhat stronger desire to eat maize or rice, when available. Many farmers said that they grew maize mainly as an income generating activity, and to a lesser extent for family consumption. The people in Nkhata-Bay only started eating maize *nsima* (dumpling-like porridge), in this century, especially after the cassava mealybug pest in 1986 that virtually destroyed the entire cassava crop along the lake-shore areas. Until then maize was predominantly eaten on the cob as a snack.

The “goodness” of cassava was expressed by many. The Tonga proverb “*Chigawo chilera walanda*,” meaning “Cassava nurtures the disadvantaged”, was commonly expressed. The advantages of farming cassava were highlighted with statements like “We do not add fertiliser to it, we just hoe, plant and weed without fertiliser”, or “Cassava is gold, it is a complete food. On top it has the relish/sauce, in the middle the planting material or firewood, and in the ground the *kondowole* (dumpling-like porridge).

*Kondowole* is the main staple dish in the district. It is made from flour obtained by pounding soaked and subsequently well dried cassava roots. The flour is mixed with boiling water to form *kondowole*. There was agreement among all the farmers interviewed that roots from bitter cassava cultivars had to be made safe for consumption by at least three days of soaking, followed by several days of sun-drying before being pounded into flour. At low temperature and overcast weather, soaking and drying require extra days.

Water for soaking cassava roots was said to be available all year round and all cassava roots, be they bitter or non-bitter, that are to be processed into flour are first soaked, and then dried. The soaking ponds, known as *mathawale* in the Tonga and Tumbuka languages, are normally constructed within 10–300 m from the homestead. A hole is dug down to the water-table and lined with a special type of grass and left to fill with water overnight. Water is changed when it is too cloudy or pungent in smell after about two weeks. Some families soak their cassava roots in clay pots, pails or cemented ponds located within the homestead. Subsequently, the roots are dried on a rack, 0.1–1.0 m off the ground, or on rocks or mats.

In all the communities women in fertile age are exclusively responsible for the entire processing of the cassava roots, whereas women with adult grandchildren and daughter-in-laws rarely engage in processing. In households without women, cassava processing might be carried out by men, but this was rarely observed. All the respondents reiterated that the potential toxicity of raw roots from bitter and toxic cassava cultivars is well known to everyone. However, the necessity to render such

roots safe through soaking and drying was seldom brought forth as a problem. Both men and women said that the most laborious part of processing was the pounding of the well dried roots into flour. The substitution of manual pounding with mechanised milling suitable for dried cassava roots was consistently stated as the main requirement for improvement of cassava processing. The few existing mechanical maize mills are not optimal for milling dried cassava roots, as the coarse flour obtained yields a sticky *kondowole*. Therefore, cassava roots are usually milled only when making a composite flour with maize.

### *Naming of Cultivars*

Based on botanical appearance the farmers could distinguish almost all cassava plants as belonging to a specific cultivar with a known local name. Each farming household grew three to ten or more different cultivars, mixed in the same fields. Women, men and children could identify whole plants as belonging to a certain cultivar and they were familiar with the names of the local cultivars. However, the women were more adept than the men at distinguishing the cultivars by name just by a rapid glance at the plant or by looking at a stem cutting or a root. The most commonly grown cultivars had the same name throughout the district, e.g. for the toxic and bitter cultivars 'Gomani', 'Tepula', and 'Koloweki' and for the non-toxic cultivars 'Mbundumali' and 'NyaChikundi'. The less common cultivars seemed to have different names in different parts of the district. Farmers sometimes classified plants from the same cultivar into subgroups, based on slight differences in morphology, for instance, 'Gomani White' and 'Gomani Black' based on the outer skin colour of the root.

The rarer plants whose origins could not be traced were usually referred to as "bird-droppings" ('Munyakayuni'). It was thought that birds may have brought in the cassava seed. Cassava is almost exclusively vegetatively propagated through stem cuttings, but farmers knew it could also be grown from a seed. Of the 54 cultivars mentioned during the study, 20 were named after the introducer or its geographical origin, 20 according

to one of its special attributes and 10 bitter and 4 cool cultivars, respectively were named in a way that we were unable to decipher (Table I). The most abundantly grown bitter cultivar, 'Gomani', was said to have earned its name from a man called Gomani who had brought the stem cuttings from Zambia to Nkhotakota district south of Nkhata-Bay. The most popular non-toxic cultivar was called 'Mbundumali', meaning figuratively "it is extremely satisfying" in the Sukwa or Ngonde language spoken in the northern tip of Malawi. 'Mbundumali' roots were much liked as a snack due to the floury starchy taste when eaten fresh, boiled or roasted.

### *Classification of Cultivars*

Besides the names for each cultivar as described above, the farmers in all the communities classified cassava cultivars as belonging to either of two groups based on whether the roots could be eaten fresh or would need to be processed before consumption. The first group was known as "cool" cultivars and the second as "bitter" (*or* "dangerous") cultivars. It was stated by the farmers that the more bitter the root tasted the higher was the risk for poisoning if eaten fresh, and conversely the less bitter a root tasted the lower the risk for poisoning regardless of what sort of cultivar it originated from. "Cool" cultivars that mainly provided roots that did not taste bitter were grown to be eaten fresh as snacks, whereas the bitter cultivars were grown for making the staple dish *kondowole*. If occasionally there was an abundance of the non-bitter roots then they could also be processed into flour. Conversely, roots from bitter cultivars that after tasting were found to be non-bitter were occasionally eaten fresh.

Although each cultivar is classified as either "cool" or "bitter" based on intended end use of the roots, the farmers stated that the taste of individual roots could be graded on a continuous scale from *very cool*, *cool*, *intermediate*, *bitter* to *very bitter*. Farmers also classify some cultivars as being "intermediate" if they sometimes provide roots that were "cool" and sometimes "bitter" in taste. By tasting the tip of a single root the farmers

TABLE I

The 40 cassava cultivars grown in the studied sections for which the meaning of the name could be identified

Name	Meaning of the name	Type of cultivar *
<b>A. Introducer</b>		
Chimpoto	one from the north	bitter
Gomani	name of male introducer	bitter
Nyamatete	name of female introducer	bitter
Makwenda	name of male introducer	bitter
Tansania	neighbouring country	bitter
Chiyuni	one of the birds	bitter
Munyakayuni	bird "droppings"	bitter
NyaPhiri	name of female introducer	bitter
Dambanjoka	name of the village of origin	bitter
NyaNkhata	name of female introducer	bitter
NyaHarawa	name of female introducer	bitter
Daeslaama	name of neighbouring country	bitter
Chilikoma	the original one from Likoma Island	bitter
NyaChikundi	name of female introducer	cool
Beatrice	name of female introducer	cool
Bitisaley	name of female introducer	cool
Lois	name of female introducer	cool
Hanocki	name of male introducer	cool
Sara	name of female introducer	cool
Bitisumani	name of female introducer	cool
<b>B. Attributes</b>		
Koloweki	one that spreads itself everywhere	bitter
Tepula	it relieves	bitter
Kamphunobi	black nose tip	bitter
Chitembwere	it is all over the place	bitter
Kanono	the hard one	bitter
Mpapa	one that reproduces a lot	bitter
Vituba	very white appearance	bitter
Chiswanthema	pot cracker	bitter
Kabalika	the one that shines	bitter
Matutumusi	one that swells up	bitter
Mpuma	the whole one	bitter
Muzghoka	one that changes	bitter

TABLE I (*continued*)

Name	Meaning of the name	Type of cultivar *
Nyamakozo	one that smells like urine	bitter
Mbundumali	one cannot finish eating it	cool
Mwaya	luck	cool
Chimpuno	the nose like one	cool
Biriwiri	bright and shiny	cool
Kasamba	the leafy one	cool
Fyoka	one that easily breaks	cool
Kamuluso	it has a reputation	cool

\* According to the broad classification of the two groups based on intended end-use of roots.

said they could discern whether a specific root was very safe, unsafe or very dangerous to consume raw. The farmers' experience was that bitter taste of a root was associated with the risk of poisoning if eaten fresh and that a root without any bitter taste was always safe to eat raw. Only one very old woman, well over 90 years of age, living in the northern part of the district, indicated that very rarely a cultivar may be encountered that provided non-bitter roots with a risk for toxic effects. However, she stated that: "We do not keep nor do we grow dangerous cassava cultivars that can harm us. For all cassava cultivars that we continue to grow we must know everything about them. If we are not able to taste the danger in a cassava cultivar then we kill it. That is to say we stop growing it."

Farmers in all the communities stated that the taste and the risk of poisoning from the roots of plants of the same cultivar co-varied with the fertility of the soil, the time of the year and the moisture level in the ground. The moisture content in the soil was indicated as the main determinant for how bitter tasting the roots would be. The farmers classification of the cultivars into "cool" and "bitter" was thus based on their experience of average taste and risk for poisoning when grown in presently available soils. Fertilisers were not applied to cassava in the area.

*Cool cultivars*, were in the Tonga, Tumbuka or Chichewa languages, referred to as *chizizila*, *chizizima*, *chachiwisi* or

*chizizira*, respectively. All these words mean some form of coolness in relation to temperature. One woman said that eating fresh roots from "cool" cultivars "is like drinking cool spring water". This group of cultivars was also referred to as calm or timid, *chizika* in Tumbuka and Tonga. Several respondents said: "Calm or timid means that it does not taste bad. It is good like a well behaved child. We can just go out in the field, up-root it and eat it raw without being afraid of being poisoned". This practice was referred to as "*tikucheta cha*" which means "*take a bite without fear*".

In Nkhata-Bay raw roots from cool cassava are well liked as a snack and used as a thirst quencher when working in the fields. The word *kucheta* was used to describe the act of tasting for bitterness and thus danger. The same word is used for savouring delicacies, in the way as wine is savoured in other cultures. Farmers stated that savouring was not considered necessary for the truly "cool" cultivars: "Unlike the roots from intermediate cultivars that we have to check for safety through tasting". In none of the interviews nor discussions were the cultivars that provide non-toxic and non-bitter roots referred to as sweet. When asked about this disparity between Tonga, Tumbuka, Chichewa and English terminology one man said: "The way you people that have gone to school in English refer to cassava roots as being sweet implies that the cassava tastes sugary like, which is not the case". This point was repeated in different sessions and none of the above mentioned words for cool cultivars mean anything remotely close to sweet.

"Dangerous" was the most common term used for cultivars that in most circumstances provided roots that were bitter and toxic and constituted a lethal hazard if eaten raw or insufficiently processed. Several words were used interchangeably in the three languages to describe the dangerous cultivars. The word *chibaya* means it can kill you, *chiheni* means it is a bad thing and *chibaba* may be translated as bitter or painful. However, the farmers were quick to point out that in this context the word *chibaba* referred more to the state of imbalance that the mouth and the whole body attained after consuming unprocessed raw bitter roots from these cultivars. The words

*chikali* and *chingakupweteka* literally mean dangerous, angry, harmful or refer to something that can potentially hurt a person. None of these words simply refer to bitter taste, but it was apparent in all the communities that the bitter taste of cassava roots was regarded as a decisive marker for danger. An explanation for this was: "cassava that is dangerous or harmful is termed as *chikali* since it tastes unpleasant or bitter, *chibaba*. In fact we equate these cultivars with fierce persons. They are capable of being dangerous, but if you know how to handle them and their fiery character then you can still live in harmony with them. When such people are very angry they can be very dangerous and are capable of killing, just like the dangerous cassava cultivars. If they are improperly processed they can injure a human being."

"Intermediate" cultivars were referred to as *vyakubabira* which means slightly unpleasant to the taste or slightly bitter. These cultivars were considered to be a sub-group of the dangerous cultivars and were also referred to as *chibabiya* (Tonga), *chikubabira* (Tumbuka) or *chowawira* (Chichewa). These words imply a hint of bitterness in taste. The taste and toxicity of these cultivars was said to vary with the environment. Such changes could result in the reclassification of a cultivar and a changed use as food, as exemplified by the following statement: "Up until last year 'Chitembwere' was known as a "cool" cultivar. We did not need to taste it before boiling and consuming it together with tea. But nowadays, it is necessary to check the taste since the rains come much later and stop much earlier, the soil fertility is declining and also because the mealybug pest of 1987 tends to make cultivars more bitter. So now we end up using the roots from this cultivar more in the making of flour than for eating it as a raw or boiled snack." Many farmers also stated that since the mealybug infestation there had been a shift in the taste of fresh roots from many cultivars towards a more bitter taste and increased risk for poisoning.

### *Preference for the Bitter Cultivars*

The larger part of cassava plants grown were stated to be cultivars of the "bitter" type and they were grown to make flour

for the staple food. Nearly all the farmers said they also grew a small proportion of “cool” cultivars in order to have roots that could readily be eaten raw as a snack. The main rationale given by both female and male farmers for wanting roots from cultivars grown for making flour to be bitter and toxic was that this characteristic improved food security. The concept of food security has a specific word, *kuvikiliya*, in the Tonga and Tumbuka languages. The reasons for preferring bitter cultivars could be grouped into three categories as shown in Table II.

*Deterring human theft* was emphasised as the major reason for growing “bitter” cultivars by both men and women farmers. Many farmers said “we would never just plant the *cool* cultivars in the fields, because then we would not have any cassava left for the staple dish, *kondowole* and we would starve”. Especially in the densely populated lake shore zone, farmers said the main problem in their community was hunger: “it is common that people plainly steal your cool cassava roots to ease their hunger, but this was not the practice in the past.” “It is mostly young men that steal from the fields. The young boys are constantly in and out of the fields looking for food, but that is due to hunger.” They contrasted such thefts from the fields to the stealing said to sometimes be done by women: “When women steal, they usually steal the roots that have been left to soak or dry”. The farmers clearly distinguished differences in the past and present thefts. “In the past if a stranger walking past your cassava fields had a hunger urge there was an acceptable cultural practice of

TABLE II

The three categories of reasons for preferring bitter cassava cultivars

Protection	Yield	End-product quality
Deters human theft	Higher yields, especially in poor soils	Whiter flour
Reduces spoilage by animals		More palatable dumpling-like porridge ( <i>kondowole</i> )
Deters families from unplanned harvesting		Tender and tastier leaf relish

harvesting someone's cool roots. He or she would uproot a plant of cool cassava that he or she needed for his or her consumption and replant the stem cutting." Theft was said to be rare or non-existent in the past, but nowadays farmers experience constant up-rooting of the cool cultivars, without any sign of replanting and sometimes they take the stem cuttings with them. It was emphasised that thefts could result in a lack of planting material for the cool cultivars: "If you are lucky you will find the stems before they have dried out so that you are able to replant the cuttings." "Cool" cultivars were also described as being *chipovu* literally meaning soft or soft-hearted. One woman said: 'Mbundumali' is simply too much of a softie ("*ni chipovu*") because it is readily stolen, rampantly harvested and the monkeys never leave it alone".

In a focus group discussion that included socially disadvantaged destitute males and females, the farmers explained that the exclusive use of dangerous cultivars for production of the staple food was a life-saving coping mechanism. "If you only or mostly plant non-bitter cassava in your field, the chances are high that you encounter a lot of stealing. If you complain you will be subjected to name calling. Especially the young men think it is their right to harvest the fruits of our labour". If and when a single woman reported a theft of cassava from her field she was often referred to in Tonga or Tumbuka as *wali na pamulomo* or *ba kuweleweta*, literal meaning "she has a big mouth" or "she is talking nonsense". The perceived lack of community action against theft was a common explanation given for preferring bitter cultivars by several informants that belonged to the lower socio-economic rungs. Women pointed out that it was virtually impossible for a female-headed household to grow a large area of cool cassava because the whole field would be invaded by unwanted "harvesters". The women went on to say that it was mostly men that had the power, that is the status, to grow large fields of cool cassava. Such male farmers often planted *juju magic* in their fields of cool cultivars. This was done by calling a diviner who performed a sacred ritual in the field so that intruders would get caught or they would have something evil happen to them when stealing. Word was spread

throughout the village that so and so's fields had *juju magic* and this deterred theft to some extent. Such farmers commonly stated that they had "closed" their field from intruders. It was observed that the few farmers mainly growing non-toxic cassava used *juju magic*, and were normally well off men who owned large pieces of land. However, many farmers also said that with the input from the missionaries and education in the area, young people no longer believed in *juju magic*. They attributed the increased incidence of stealing to the changes in traditional and cultural beliefs.

*Reduced destruction by animals* was mentioned as another major reason for preferring to grow bitter cultivars. In the lakeshore zone, spoilage by animals was negligible, in the escarpment zone it was more pronounced, and in the mountainous plateau zones it was stated as a severe problem. Plant spoilage was mainly done by wild pigs (*nguruwe*), black and white monkeys (*amunkwere*), baboons (*apusi*), moles (*amizumi*) and domestic animals such as goats (*mbuzi*). Farmers reported that animals were very clever and they selectively uprooted the cool cultivars only, but when in doubt they could uproot a lot of plants until they found those that were edible: "There are so many monkeys in this area these days. We have to guard our cassava day and night." In the problematic areas, farmers expressed that spoilage by animals of the cool cassava cultivars was so grave such that it was inconceivable to grow the non-dangerous cultivars in any significant proportions. Many farmers stated that they would be helped if the animals were killed: "In the past the forest rangers routinely shot the animals, but this was no longer the case due to the lack of ammunition". The farmers claimed that this had led to an uncontrolled increase in the vermin population.

*Deterring unplanned harvesting.* Farmers were in agreement that the cultivation of dangerous cultivars also reduced the social obligation of sharing fresh roots with visitors. Since it was not possible to snack on raw bitter roots families could truly apologise for not having any cool roots to share with visitors, as often mentioned to the investigators: "if only we had some cool

cultivars we could have offered you something to chew on". It was also very apparent that rampant harvesting of the cool cultivars by the children within the family was a problem. Even in areas where both human theft and spoilage by animals were negligible or non-existent, farmers were very emphatic about growing dangerous cultivars for food security. Farmers said that especially in the months of September to December when food stores are low, youngsters would carry out unplanned piece-meal harvesting, known as *kusappuwa*, of roots from the "cool" cultivars: "There is too much *kusappuwa*, when it comes to the "cool" cultivars".

The fact that women were responsible for the processing was perceived by the women farmers as an assurance of household food security. The necessity to exhaustively process the bitter roots for safe consumption, enabled the women to decide independently from their husbands and children when and how much to harvest of the bitter cultivars. Many statements by the women, and also by several male informants, explained that toxic roots from bitter cultivars empowered the woman in the household to decide when to harvest such cultivars. It was inconceivable for the farmers that the family would only plant non-dangerous cultivars, since the temptation to sell fresh roots would be too large. The reason for the temptation was that roots from cool cultivars could be converted into cash on the same day. Many said: "We will just finish up-rooting the roots needlessly and then what would we have to eat".

*High yields* was stated as another major reason for growing the bitter and toxic cultivars. They were said to yield a little more than the cool cultivars, especially under adverse climatic conditions and on poor soils. According to both male and female farmers, the fact that bitter cultivars could be left in the ground for a much longer period than the cool ones without any serious damage to the yield was a big advantage. The roots from bitter cultivars were bigger than those from the cool ones. It was generally preferred that the roots from the cool cultivars be smaller in size since they were used as snacks and had to be easy to peel and to chew. However, there was a limit as to how small the root should be in size.

*Whiter flour and more palatable kondowole* was also given as a preference for bitter cultivars by women farmers. They said that flour from *bitter* cultivars was superior to that made from *cool* cultivars. The dumpling-like porridge made of flour from bitter roots was known as less sticky and much easier to mould with the fingers. Superior end-product quality was supported by such statements: "The *kondowole* made from bitter cassava roots, especially the cultivar 'Tipula', is very white and it tastes so much better." The quality of the flour was also said to depend very much on the season. Although the roots were reported to be more bitter during the dry hot season the women unanimously stated that the best *kondowole* was obtained in this period.

*Cassava leaves* were locally known as *mayani* or *chigwada* and are abundantly eaten as a relish together with the *kondowole*. The taste and tenderness of the cooked cassava leaves are extremely important for the women. The relish is made by first pounding the cassava leaves to small bits and then boiling them with salt, and available condiments for about 30 to 60 minutes or longer, depending on the age of the leaves. It was generally accepted that one did not need permission to gather cassava leaves from a neighbour's field as long as it was not excessive pruning. The leaves were prepared with bicarbonate of soda or in the traditional way with a filtrate of the ashes of cassava stems, bean vines, pumpkin vines or water reeds. Most women had also observed that the leaves from the dangerous cultivars were much more tender *kuwolowa* when cooked. Many of the women believed that it was the bitter sap that made the leaves from bitter cultivars more tender than the cool ones. Some women furthermore, said that cassava leaves with mosaic disease were more tender and tastier than the non-diseased.

## DISCUSSION

The main findings of this study were: (1) Farmers have an elaborate knowledge of the cassava cultivars that they grow based on a folk taxonomy that uses the botanical appearance of

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plants. Bitter taste was used as an indicator for toxicity and bitter roots were always processed by soaking before consumption largely in the form of the staple food *kondowole*. (2) Bitter and cool cassava cultivars can be regarded as two crops since they have two very distinct roles in the farming and food system. (3) Farmers preferred that cultivars grown for the purpose of *kondowole*, the staple food, should have bitter roots because this aspect provided protection against theft, destruction by animals and unplanned harvest by family members.

### *Farmers Knowledge*

An important finding was that farmers could distinguish each and every cassava plant as belonging to a specific cultivar based on its botanical appearance. Cassava cultivators seemingly select cultivars using a combination of factors that will enable them to characterise a cultivar without observing and remembering the performance of each plant. However, in accordance with the findings of Boster (1985) in South America, some informants confused some cultivars that were most similar to each other. This was not the case with some cassava farmers that took it upon themselves to keep and know many cassava cultivars in their fields, as also found in communities in South America (Boster, 1984; 1985; Brush, Carney and Huaman, 1981; Salick, Cellinese and Knapp, 1997). We also found that women farmers were more adept than the men in distinguishing the cultivars and in identifying specific advantages regarding the end-product outcome. The standardised naming within the farming system and the fact that most names denoted origin or some special attribute of each cultivar exemplifies that the wealth of indigenous knowledge about the cultivars grown by these African farmers is as extensive as that which is documented among Indian tribes in South America (Brush, Carney and Huaman, 1981; Boster, 1984; Dufour, 1994; Salick, Cellinese and Knapp, 1997). This extensive local knowledge about cassava cultivars in Africa where the crop is relatively new, is supported by a sample survey across Africa (Nweke and Bokanga, 1994). Cassava breeding and extension services in Africa should be

based more on existing knowledge and preferences regarding cassava cultivars (Richards, 1985).

### *Bitter and Cool Cassava Cultivars are Different Crops*

The farmer's statements that bitterness and toxicity co-varied on a continuum from very bitter to very cool, depending on the type of cultivar and environment is in accordance with results of experimental studies (de Bruijn, 1973; Bokanga *et al.*, 1994). The relevance of the practice to test if a root can safely be eaten fresh or directly boiled by tasting its tip for bitterness is also supported by the experimental findings of association between bitterness and toxicity (Sundaresan, Nambisan and Easwari-Amna, 1987).

In spite of the farmer's statements of a continuous biological variation of taste and toxicity in cassava roots we found that they regarded cassava as two different crops in their farming and food system. This can be compared to communities where plantains are eaten as a staple food and bananas as a snack where clearly, plantains and bananas constitute different crops although the plants look similar. By regarding cassava as two crops it is easy to understand why farmers want roots from cultivars grown to provide flour for the staple food *kondowole* to be bitter and toxic, and cultivars grown to provide fresh roots for snacks to be free from potential toxicity and bitterness. The preferred attributes differed considerably between bitter cultivars grown for flour production and cool cultivars grown for consumption in the raw form. Different breeding targets for levels of cyanogenic glucoside and taste must therefore be set for these two types of cultivars.

### *Protection against Theft*

Protection against human theft believed to be mainly done by young men with acute pangs of hunger ultimately emerged as the dominant reason for preferring bitter cultivars in Nkhata-Bay. The reasons given as to why bitterness deterred the young men from stealing these particular cultivars made sense. Bitter roots were not attractive for a hungry person since processing

required one week before the roots could be eaten. Furthermore, carrying out processing would put a male thief at risk of being discovered, since processing is predominantly done by women. Finally, there was no point in stealing toxic roots to sell, since there was a very limited market for un-processed bitter cassava roots.

The issue of theft was a delicate topic to discuss and this may explain why we have not found any published reports on protection against human theft being a reason for cassava farmers wanting their cultivars to provide toxic roots. However, anecdotal information from several cassava growing communities with food insecurity suggests that protection against human theft may be a much more common reason for preferring toxic and bitter cassava cultivars than has been previously realised (Kapinga *et al.*, 1997). Studies by Salick *et al.* (1997) also showed that cassava cultivators would go as far as stealing to obtain planting material of certain types of cultivars. These delicate issues may have also been missed in this study, had not trustful relations been established between the investigators and the informants (Cassidy, 1994).

Our inclusion of communities from different agro-ecological zones of the district and the consistency of the findings, makes it reasonable to extrapolate the findings to the whole district and to similar districts along Lake Malawi. The fact that food insecurity was the underlying reason for preferring roots grown as a staple food to be bitter, suggests that similar preferences may be revealed in many other cassava farming systems in Africa if they were to be studied with qualitative methods (Dawson *et al.*, 1993; Morse and Field, 1995).

The revelation that the protective effect of toxicity against theft was especially important for households headed by destitute women, is a new finding and of special importance for the prevention of cyanide exposure from insufficiently processed cassava. The reasons being that these food insecure households have both the strongest preferences for growing the bitter cultivars and are most prone to take short-cuts in processing that results in dietary cyanide exposure (Banea *et al.*, 1992; Tylleskär *et al.*, 1992). Nevertheless, promotion of food security

and safe processing seems to be a more feasible way to prevent toxic exposure than to try and convince destitute women to grow non-toxic cultivars.

### *Protection against Vermin*

The explanations given by farmers for why bitterness and toxicity of cassava deters animals from eating the roots are in accordance with the biological understanding of cassava cyanogenesis. The importance of protecting cassava fields from wild animals has earlier been reported from Nkhata-Bay district (Msukwa and Pelletier, 1990). Statements by farmers that toxicity and bitterness deter wild animals from eating cassava have also been reported from other parts of Africa (Essers *et al.*, 1992) and South America (Purseglove, 1968). In this context, it is worthy noting that the edible parts of other staple crops are reproductive organs that mature rapidly during some weeks before the fixed harvest time, and these cereals and tubers can be stored unprocessed for prolonged periods at the homestead where they are guarded. In contrast, cassava is the only major staple crop where the edible part is a storage organ with several months of starch accumulation and no fixed harvesting time. Unprocessed cassava roots can not be stored but the roots can be left in the ground for months or years thereby providing food security. This prolonged availability of starchy roots in cassava fields can explain why protection against attacks by wild animals and thieves is more crucial for cassava than for other staple crops that when necessary can be guarded day and night for just a few weeks prior to harvest.

### *Empowerment of Women*

Even in the absence of spoilage by animals and human theft, families stated that they would never only grow cool cassava. Given that cassava processing is entirely a woman's domain meant that women were in control of the crop. Contrary to what has been assumed about cassava cyanogenesis and women's high labour input in processing, the toxicity of cassava roots

empowered women. This somehow strange gender effect of cassava toxicity shows that people adjust to hardships with measures that ensure their survival (Ohadike, 1981). It is therefore logical to understand that bitterness also deters family members, other than the women in charge, in both poor and better off households from unplanned harvesting. The temptation to sell cool cassava roots might be greater for the men since a man's success in the studied area is judged by his ability to provide purchased food and goods for the family.

As found in other studies (Nweke, 1994; Dufour, 1989; Nye, 1991), the farmers not only process cassava for detoxification purposes but also to acquire the desired quality and texture of the products. They did not regard bitterness and toxicity as a problem as all roots used to make flour, be they bitter and toxic or not, had to be similarly processed. The women emphasised that the time and energy spent on pounding roots was the most painstaking post-harvest activity related to cassava. An acyanogenic cultivar, with all other characteristics similar, would thus not reduce their workload, but the provision of mills would significantly reduce their workload.

### *Bitterness and Yield*

The farmer's statements that bitterness and toxicity are associated with higher yield is supported by other studies of cassava farming systems in Africa (Nweke and Bokanga, 1994) and South America (Nye, 1991), but not with experimental studies (Mahungu, 1994). This difference may be explained in two ways. First by a direct positive impact on yield of high levels of glucosides through some unknown biological mechanism operating under harsh conditions, like nitrogen store or disease protection. Secondly, if bitter cultivars have been selected for high yields for many centuries this will have resulted in a coupling of yield and bitterness in local bitter cultivars. Good taste may have been a more important selection criteria than yield for cool cultivars. The coupling between yield and bitterness in local cultivars may be lost when germplasm is collected from a sub-continent and crossed in breeding programmes.

## CONCLUSION

We find the farmer's rationale for preferring bitter cultivars sound from both a biological and social perspective, when considering their precarious livelihood and perpetual food insecurity. In several respects, the situation of the studied population may be similar to that of the farmers in the Amazonia that domesticated cassava and discovered how to effectively remove cyanogens through processing. It may therefore be hypothesised that cyanogenesis favoured the domestication of cassava. Without the unique protection offered by cassava cyanogenesis against vermin and thieves the storage roots of cassava might never have become a major staple crop for mankind. The reason being that the storage roots of cassava are vulnerable to vermin and theft during a much longer period than are the edible parts of cereals and tubers that are fast maturing reproductive organs. Furthermore, this paper demonstrates the wealth of indigenous knowledge based on folk-taxonomy and thus the need to base plant breeding on farmers' preferences.

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# Paper II



## Low dietary cyanogen exposure from frequent consumption of potentially toxic cassava in Malawi

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In a cassava-growing area in Malawi, where roots are processed by soaking and water is available throughout the year, we interviewed 176 women farmers regarding their preferences for cassava cultivars and frequency of cassava consumption. Dietary cyanogen exposure was estimated from urinary levels of linamarin, the cyanogenic glycoside in cassava, and urinary thiocyanate, the main cyanide metabolite. Protection against unplanned harvest by family members, theft and animal spoilage were stated to be very important reasons for growing bitter cassava cultivars by 91%, 90% and 74% of the women, respectively. The mean ( $\pm$ SD) number of cultivars grown by each woman was 4.6 ( $\pm$ 2.4). The correlation between mean taste and mean danger scores for the 25 most grown cultivars was strong ( $r > 0.98$ ). The scoring indicated that cultivars belonged to two distinct groups, eight to a group referred to as 'cool' and 17 to a group termed 'bitter'. The dumpling-like porridge (*kondowole*) made from cassava flour from bitter roots was eaten twice daily by 51% and at least weekly by 81%. The mean ( $\pm$ SEM) urinary linamarin was 14 ( $\pm$ 1)  $\mu$ mol/L and thiocyanate was 50 ( $\pm$ 4)  $\mu$ mol/L, less than a tenth of levels reported from populations eating insufficiently processed bitter cassava roots, and in the same range as in a non-smoking Swedish reference population. We conclude that cyanogenesis is a preferred characteristic of cassava by the studied farmers because it enhances food security. The availability of water and their knowledge about toxicity and processing enables these women farmers to provide a safe staple food from bitter cassava roots.

### Introduction

The release of cyanide from stored cyanogenic glucosides in the roots and leaves of the cassava plant (*Manihot esculenta* Crantz), through the action of an endogenous enzyme in the plant

cells, is known as cassava cyanogenesis (Koch *et al.*, 1992; Bokanga, 1995). The levels of cyanogenic glucosides, mainly linamarin, in plant tissue depend on both genetic and envi-

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ronmental factors and the degree of bitterness of the root parenchyma is positively associated with the level of linamarin (Sundaresan *et al.*, 1987). During processing, such as soaking of roots that results in disintegration of the plant cells, the linamarin is first enzymatically hydrolysed and the acetone cyanohydrin formed breaks down to hydrogen cyanide that evaporates during subsequent drying. These three compounds are jointly known as cyanogens, and they can be reduced to negligible levels by various traditional processing methods first developed by the Amerindians thousands of years ago (Dufour, 1989; Nye, 1991).

Dietary cyanide exposure from the consumption of ineffectively processed cassava may result in acute poisonings and it is implicated as a causal factor in some other toxico-nutritional diseases (Rosling & Tylleskär, 1995). However, these diseases have only been reported from rural communities with concomitant severe food insecurity and malnutrition. The high dietary cyanide intake results from short-cuts in cassava processing induced by food shortage and intensive sales (Tylleskär *et al.*, 1991). It seems paradoxical that farming communities where these toxic effects occur seem especially keen on growing bitter and toxic cultivars (Essers *et al.*, 1992). Qualitative interview studies in Tanzania (Kapinga *et al.*, 1997) and Malawi (Chiwona-Karltun *et al.*, 1998) indicated that cassava farmers regard bitterness and toxicity of roots as a protector of their cassava against theft, spoilage by animals and unplanned harvesting by family members. The area studied in Malawi has water available throughout the year for the soaking of cassava roots and the farmers stated that they had no problems with intoxication. Cassava cultivars were classified into two groups: 'bitter' needed processing before consumption whereas roots from 'cool' cultivars could be eaten fresh or directly cooked. To verify the findings of the qualitative study (Chiwona-Karltun *et al.*, 1998) we conducted a quantitative study of the farmers' cultivar preferences and of the safety of the cassava consumed.

The first aim of this study was to estimate the importance attributed to each of the different reasons for growing bitter and toxic cassava cultivars and to elucidate the types and advantages of cassava cultivars planted through

structured interviews with a sample of women farmers. The second aim was to quantify the consumption frequency of cassava products by these women and to assess their dietary cyanogen intake by measuring urinary levels of linamarin and thiocyanate, the main metabolite of cyanide.

## Study area

Nkhata-Bay district is situated along the shore of Lake Malawi in northern Malawi and has 140 000 inhabitants. Farming is the main livelihood and cassava occupies more than 70% of the cultivated land. It has been the prevailing staple food for more than a century (Crosby, 1980; Musukwa & Pelletier, 1990; Paris, 1991). Throughout the year cassava roots are largely processed into flour by soaking for 3 to 7 days, followed by sun-drying and pounding into flour. The flour is used to prepare the staple food *kondowole*, a dumpling-like thick porridge that is mostly eaten together with a stew made of cassava leaves, fish, legumes or pulses (Chiwona-Karltun *et al.*, 1998). The agricultural extension service divides the district into 53 sections. Two sections situated 40 km south of Nkhata-Bay district centre were selected for this study as being representative of the densely populated agro-ecological zone along the Lake shore (Chiwona-Karltun *et al.*, 1998). Each section comprised eight blocks with about 100 households. One block, reported to be typical for the agricultural and social situation, was selected for the study from each section. 'Thowolo-B' in Lweya section at the Lake shore north of Chintheche and 'Matyenda-1' in Mgododi section 2 km inland from Chintheche trading centre (Chiwona-Karltun *et al.*, 1998).

## Subjects and methods

The study was conducted in July and August 1996 and it was approved by the Research Division and Extension Service of the Ministry of Agriculture, the District Health Commissioner in Nkhata-Bay, and by the community leaders of the study areas. Following informed consent, a house-to-house census identified 98 households in 'Thowolo B' and 102 in

'Matyenda-1'. The woman in charge of cooking in each household was interviewed in the most appropriate of the three local languages using a structured questionnaire. Thirteen households were excluded since they had no woman in charge of cooking, and two because the woman in charge was away from home. Two declined participation and seven were excluded because of inadequate urine volume. The remaining 176 households were included in the study.

The questionnaire included questions on age, household size, marital status and individual wealth-ranking based on the household's ability to secure food and essential commodities all year round. Women were asked to grade the importance that they gave to eight identified reasons (Chiwona-Karlton *et al.*, 1998) for growing bitter cassava, to name all the cultivars grown, state the main advantage of each cultivar and to score the average taste of the root of each cultivar grown on a categorical scale ranging from very cool (1), cool (2), intermediate (3), bitter (4), very bitter (5) and to very very bitter (6). They were also asked to estimate the average danger, i.e. risk of acute poisoning from eating fresh roots, of each cultivar grown using a danger scale ranging from no danger (1), somewhat dangerous (2), dangerous (3), very dangerous (4) and very very dangerous (5). The terms used for each taste as well as danger score were well-established expressions in the local languages.

In addition, each woman was asked about frequency of consumption during the past 7 days of the five main cassava products and other food items in season, as previously identified (Chiwona-Karlton *et al.*, 1998). The frequency options were three times, twice or once per day; four to six or one to three times

per week; or never. One bereaved woman that did not complete the food frequency questionnaire was excluded from the dietary analysis and one woman's vague answer on the maize consumption was regarded as no consumption. At the end of the interview, each woman was asked to provide a urine sample in a 20 ml plastic bottle with five drops of 10% thymol in iso-propanol added as a preservative. The urine was frozen the same day and transported frozen for the determination of linamarin (Carlsson *et al.*, 1995) and thiocyanate (Lundquist *et al.*, 1995) at Uppsala University Hospital, Sweden.

## Results

### Women interviewed

The mean  $\pm$  SD age of the 176 women was  $40 \pm 15$  years, the range was 15 to 70 years (Table 1). A total of 97 were wives to the head of the household, 68 were themselves the head of households, and 11 had other family status, for example daughter, aunt or kin of some other kind. The proportion of women that were head of households increased with age (Table 1). Five women lived alone and the largest household comprised 13 members. In a simple self-evaluation 134 women ranked their households as belonging to the 'lower', 41 to the 'middle' and one to the 'higher wealth group'. Ten of the women were teachers and the remaining 166 mainly worked with agriculture.

### Cassava cultivars

Womens' estimation of the degree of importance of each reason for growing bitter cultivars is given in Table 2. All 176 women interviewed

**Table 1.** Social characteristics of the 176 women interviewed

	Age groups			Total (n = 176)
	$\leq 35$ years (n = 72)	35–49 years (n = 47)	$\geq 50$ years (n = 57)	
Mean household size $\pm$ SEM	$4.8 \pm 0.2^a$	$6.9 \pm 0.4^b$	$5.5 \pm 0.4^a$	$5.5 \pm 0.2$
Head of household (%)	8% <sup>a</sup>	45% <sup>b</sup>	72% <sup>c</sup>	68 (38%)
Classified as lower wealth group (%)	71% <sup>a</sup>	79% <sup>a</sup>	81% <sup>a</sup>	134 (76%)

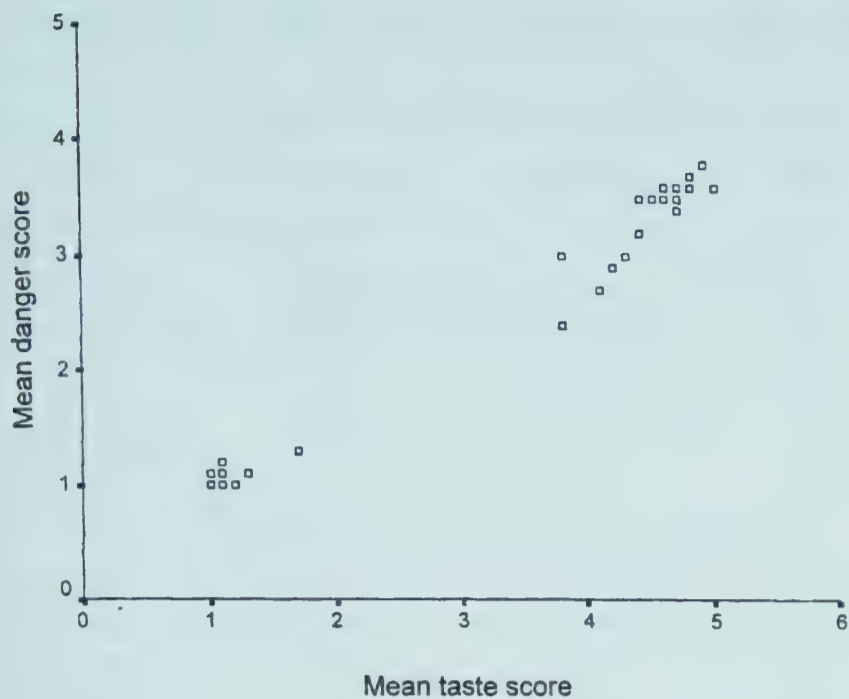
Means followed by different letter are significantly different from each other by Student's t-test and chi-square, respectively ( $P < 0.01$ ).

**Table 2.** Number (%) of the 176 women that gave each degree of importance to the eight identified reasons for growing bitter types of cassava cultivars

Reasons	Degree of importance			
	Very important	Important or less important	Not important	Do not know
Protection against:				
unplanned harvest	160 (91)	2 (1)	5 (3)	9 (5)
theft	158 (90)	2 (1)	6 (3)	10 (6)
animal spoilage	130 (74)	6 (3)	25 (14)	15 (9)
High yield	159 (90)	3 (2)	1 (1)	13 (7)
Better product quality of:				
flour	41 (23)	5 (3)	18 (10)	112 (64)
kondowole	33 (19)	7 (4)	22 (12)	114 (65)
soaked and roasted roots	34 (19)	0 (0)	1 (1)	141 (80)
leaf relish	91 (52)	5 (3)	23 (13)	57 (32)

**Table 3.** Number of women growing each of the 25 most common cultivars, the mean ± SEM of danger and taste scores attributed to each cultivar, and the percent of women growing each cultivar mentioning each advantage for growing the particular cultivar

Type and name	Number growing	Mean ± SEM danger score	Mean ± SEM taste score	Percentage mentioning each type of advantage for growing the cultivar				
				High yield	Early maturing	Good kondowole	Good snack	Other advantages
Bitter								
Gomani	122	3.5 ± 0.1	4.7 ± 0.1	40	21	18	0	21
Depweti	113	3.2 ± 0.1	4.4 ± 0.1	49	33	10	0	8
Koloweki	76	2.7 ± 0.1	4.1 ± 0.1	42	0	33	0	25
NyaHarawa	52	2.9 ± 0.1	4.3 ± 0.1	52	18	23	0	7
NyaNkhata	37	3.5 ± 0.1	4.6 ± 0.1	65	11	22	0	2
Ng'wenyani	27	3.5 ± 0.1	4.7 ± 0.1	52	14	21	0	13
Nyamakozo	23	3.7 ± 0.1	4.8 ± 0.1	74	13	9	0	4
Mpuma	20	3.1 ± 0.3	3.9 ± 0.4	30	20	20	20	10
Palamu	20	3.8 ± 0.1	4.9 ± 0.1	70	10	0	0	20
Chakubaba	16	3.6 ± 0.2	4.7 ± 0.1	63	0	18	0	19
Kanonono	13	2.4 ± 0.2	3.8 ± 0.2	67	0	25	0	8
Munyakayuni	11	3.5 ± 0.3	4.7 ± 0.2	55	0	36	0	9
Kawalika	10	3.6 ± 0.4	4.6 ± 0.4	40	30	10	0	20
Mzumara	10	3.6 ± 0.2	5.0 ± 0.0	50	40	10	0	0
Thipula	8	3.5 ± 0.3	4.4 ± 0.3	51	0	39	0	10
Nyasungwi	7	3.6 ± 0.3	4.7 ± 0.2	57	29	0	0	14
Mbayani	6	3.0 ± 0.5	4.3 ± 0.3	33	0	50	0	17
Cool								
Mbundumali	92	1.0 ± 0.0	1.0 ± 0.0	0	0	0	96	4
Chimpuno	31	1.0 ± 0.0	1.1 ± 0.1	0	0	0	97	3
NyaChikundi	31	1.0 ± 0.0	1.1 ± 0.1	0	0	0	100	0
Fyoka	15	1.1 ± 0.1	1.3 ± 0.2	13	0	0	73	14
Kweti	7	1.3 ± 0.3	1.7 ± 0.5	14	0	14	57	15
Mwaya	3	1.0 ± 0.0	1.0 ± 0.0	0	0	0	100	0
Kamwala	2	1.0 ± 0.0	1.0 ± 0.0	0	0	0	100	0
Katchamba	2	1.0 ± 0.0	1.0 ± 0.0	0	0	0	100	0



**Figure 1.** Correlation between the mean taste score and the mean danger score for the 25 most grown cultivars stated by the women interviewed for each cultivar that they grew.

were active farmers and only 5 (3%) did not cultivate cassava, 51 (29%) cultivated one to three, 91 (52%) four to six and 29 (16%) grew seven to fourteen cultivars. The median number of cultivars grown was four, and the mean ( $\pm$ SD) was  $4.6 \pm 2.4$ . A total of 45 different name-given cultivars were reported grown by the women interviewed, and of those, 25 cultivars were reported grown by more than one woman (Table 3). Another 20 cultivars were reported to be grown by one individual woman only, ten of those cultivars were classified as 'bitter' and ten as 'cool'. Finally, one or more cultivars identified as 'unknowns' were reportedly grown by 16 women.

Table 3 shows the mean  $\pm$  SEM of taste and danger scores given for the 25 most grown cultivars. The taste scores ranged from very cool (1) to very very bitter (6) and the danger scores ranged from no danger (1) to very very dangerous (5). The mean of danger and taste scores attributed to each cultivar revealed that all the cultivars fell into either of two distinct groups, 'bitter' or 'cool'. For the eight cultivars designated as 'cool' the mean taste ranged from 1.0 to 1.7, and mean danger from 1.0 to 1.3. For those designated as 'bitter' the mean taste ranged from 3.8 to 5.0, and mean danger from 2.4 to 3.8. There was a strong correlation,  $r > 0.98$  between mean taste and danger scores for these 25 cultivars (Figure 1). Table 3 also shows that the pattern of reported main advantages differs strikingly between the 'bitter' and 'cool'

cultivars, with the only slight exception being the cultivars 'Mpuma' and 'Kweti'. The two latter cultivars were scored as either being very cool, cool, or very very bitter in taste, or scored as no danger to very very dangerous. It appears as if each of these two names were used for two different cultivars.

#### *Dietary assessment*

The food consumption frequency during the last 7 days for the 175 women responding are presented in Table 4. Rice, sweet potatoes and yams were mostly reported as being eaten for breakfast meals. A total of 55% of women interviewed reported daily consumption of *kondowole* during the past week. Many women stated that the frequency would have been higher had not excessive rains earlier in the year destroyed cassava fields to the degree that the community had been declared a disaster area entitled to maize distribution. Almost all the farmers had cassava growing in the fields at the time of the interviews, but most roots had not reached maturity. The mean age was significantly higher for women eating cassava daily than for those eating it less frequently, and a significantly higher proportion of women were heads of households and ranked themselves as belonging to the lower wealth group among those consuming *kondowole* daily (Table 5).

Only three of the women interviewed openly admitted to ever having experienced acute poisonings that they attributed to bitter cassava. The perceived reasons for the acute cassava poisonings given by all three women were shortened soaking or drying time, and requirements for longer soaking times in the cold season. A total of 63 (36%) women said that they had seen or heard of others in the community having had acute poisonings after consumption of ineffectively processed bitter cassava. The farmers' experiences of poisonings were attributed to lack of time when processing the cassava roots. It was stated that in times of need some of the women would practice partial removal of the soaked 'ready' roots to obtain flour for the family.

#### *Urinary analysis*

Urinary concentration of linamarin ranged from 3 to 39  $\mu\text{mol/l}$  and the median was 12  $\mu\text{mol/l}$ . Urinary thiocyanate ranged from 2 to 410

**Table 4.** Number (%) of the 175 women interviewed that report each frequency of consumption of the main foods during the last week

Food item	≥ 2/day	1/day	4–6/week	1–3/week	Never
Cassava root products					
Kondowole (staple food)	90 (51)	7 (4)	13 (8)	31 (18)	34 (19)
Soaked and roasted	1 (1)	6 (3)	5 (3)	72 (41)	91 (52)
Raw ‘cool’	0 (0)	3 (2)	10 (6)	83 (47)	79 (45)
Boiled ‘cool’	0 (0)	3 (2)	1 (1)	42 (24)	130 (73)
Roasted ‘cool’	0 (0)	0 (0)	3 (2)	5 (3)	167 (95)
Other foods					
Maize nsima	58 (33)	9 (5)	21 (12)	31 (18)	56 (32)
Rice	2 (1)	29 (17)	22 (13)	75 (43)	47 (27)
Yams	1 (1)	2 (1)	1 (1)	40 (22)	131 (75)
Sweet potato	9 (5)	65 (37)	32 (18)	39 (22)	30 (17)
Green leafy vegetables	12 (7)	32 (18)	40 (23)	72 (41)	19 (11)
Bananas	5 (3)	9 (5)	17 (10)	78 (44)	66 (38)
Beans	0 (0)	3 (2)	7 (4)	40 (23)	125 (71)
Big fish	4 (2)	3 (2)	11 (6)	55 (32)	102 (58)
Medium fish	10 (6)	22 (12)	23 (13)	73 (42)	47 (27)
Small fish	3 (2)	16 (9)	31 (17)	73 (42)	52 (30)
Lake fly	0 (0)	2 (1)	5 (3)	77 (44)	91 (52)
Meat/poultry	0 (0)	1 (1)	2 (1)	81 (46)	91 (52)
Groundnuts	0 (0)	2 (1)	8 (5)	72 (41)	93 (53)
Cooking oil	10 (6)	8 (5)	30 (17)	18 (10)	109 (62)

μmol/l and the median was 32 μmol/l and 23 women had thiocyanate levels above 100 μmol/l. The three women that admitted to having experienced acute effects from short-cut processing had thiocyanate levels of 60, 118 and 410 μmol/l, respectively. Mean urinary thiocyanate was significantly higher than mean urinary linamarin by both paired and unpaired *t*-test. There was no correlation between urinary

thiocyanate and linamarin concentration (*r* = 0.06). Mean thiocyanate was significantly higher among those eating *kondowole* than in those not having eaten it in the last week (Table 5). Regression analysis of the number of meals of *kondowole* per week as an independent variable showed a positive association with urinary thiocyanate that increased with 1.6 μmol/l per meal (95% confidence interval

**Table 5.** The mean age and household size of the different *kondowole* consumption frequencies were compared by *t*-test at the 95% significance level. Means followed by the same letter are not significantly different from each other

	Kondowole consumption frequency			
	Daily <i>n</i> = 98	Weekly <i>n</i> = 44	Never <i>n</i> = 33	Total <i>n</i> = 175
Mean age	44.6 ± 1.5 <sup>a</sup>	32.5 ± 1.8 <sup>b</sup>	37.4 ± 2.7 <sup>b</sup>	
Mean household size	6.0 (±0.3) <sup>ac</sup>	4.8 (±0.3) <sup>b</sup>	5.4 (±0.5) <sup>bc</sup>	
Head of household	47 (48%) <sup>a</sup>	10 (23%) <sup>b</sup>	10 (30%) <sup>c</sup>	67 (38%)
Low wealth status	80 (82%) <sup>a</sup>	33 (75%) <sup>a</sup>	20 (60%) <sup>a</sup>	133 (76%)
Thiocyanate μmol/l	57 (±7) <sup>a</sup>	48 (±6) <sup>a</sup>	29 (±5) <sup>b</sup>	50 (±4)
Linamarin μmol/l	14 (±1) <sup>a</sup>	13 (±1) <sup>a</sup>	13 (±1) <sup>a</sup>	14 (±1)

Means followed by different letters are significantly different from each other by chi-square or *t*-test (*P* < 0.01).

0.2–3.0), but there was no similar association with linamarin. This did not change in a multiple regression analysis including all five cassava food products as independent variables. No other association was found between the frequency of consumption of any food item with urinary thiocyanate nor linamarin.

## Discussion

Limited understanding of cassava cyanogenesis has resulted in conflicting opinions about strategies for cassava research and promotion, ranging from regarding cyanogenesis as a reason to discourage the use of cassava, to cyanogenesis being an advantageous pesticide. It has been argued that biotechnology should be used to remove cyanogenesis from cassava to protect consumers from toxic effects (Dixon *et al.*, 1994). However, studies from the Amazon basin indicate that cassava farmers for thousands of years have conscientiously chosen to grow cultivars with bitter roots and high levels of linamarin and hence subjected themselves to lengthy processing methods to remove cyanogen substances before consumption (Dufour, 1989; Nye, 1991). This made us suspect that cyanogenesis may provide more advantages than disadvantages in many farming and food systems. The results obtained through structured interviews in the present study are consistent with earlier findings using qualitative interview methods. Cyanogenesis ameliorates household food security. Women control potential toxicity of cassava roots through their knowledge of the genetic character of cassava cultivars, the association between taste and toxicity, and processing methods for the removal of toxic substances.

### *Bitterness and toxicity are associated*

Bitter taste in cassava roots has been shown to be positively associated with levels of cyanogenic glucosides (Sinha & Nair, 1968; Sundaresan *et al.*, 1987) but some studies have only shown a weak association (Bokanga, 1994). In the present interview study precautionary measures were taken not to let the women's scoring of taste influence their scoring of toxicity, i.e. the risk of dying from acute poisoning after eating unprocessed roots from each cultivar. A six-point scale was used for scoring taste and a five-point scale for danger scoring, thereby

avoiding direct coupling of taste and danger scores. The local linguistic expressions of scoring taste and danger were also completely different. In spite of this attempt to document different experiences of taste and toxicity of the cultivars grown, we found a very strong correlation ( $r > 0.98$ ) between mean taste and mean danger scores for the 25 most common cultivars (Figure 1). This supports the recent finding that 'linamarin is the sole contributor of bitterness in the parenchyma of cassava roots', although several substances may modify the bitterness of the cortex (King & Bradbury, 1995).

### *'Cool' and 'bitter' cultivars are different crops*

Our previous qualitative interview study in the same district (Chiwona-Karlton *et al.*, 1998) indicated that the local ethno-classification denoted cultivars as belonging to one of two groups based on intended end-use as also found in other areas (Coursey, 1973). The results of this study confirm that all the cultivars grown in northern Malawi fall into either a 'bitter' or a 'cool' group when sorted according to mean danger and taste scores. Contrary to the universal use in literature of the term 'sweet' for cassava cultivars with roots that are non-bitter and have low glucoside levels, we find it prudent to use the direct translation of the term 'cool' that has been found to be widely used in both Malawi (Chiwona-Karlton *et al.*, 1998) and Tanzania (Kapinga *et al.*, 1997). The reason for this is that a better reporting of the ethno-classification and terminology used for cultivars in various cassava-farming systems may further the understanding of farmers' preferences. That the division into two groups in Malawi is based on intended end-use, is emphasised by the distribution of the main advantages expressed for each cultivar. Roots from the 'cool' cultivars are good for snacks and the 'bitter' are good because of high yield, early maturing and being good for making flour for the staple food *kondowole*. The major advantages stated for the 'bitter' cultivars are not mentioned for the 'cool' cultivars, as was also reported from South America (Purseglove, 1968; Nye, 1991; Salick & Cellinese, 1997). In both South America and Africa it seems that in most areas where cassava roots are a staple food, bitter and toxic cultivars dominate (Purseglove, 1968; Lancaster & Ingram, 1982). A survey in six

cassava-growing countries in Africa showed that 'bitter' cultivars were liked for processing into staple food whereas 'non-bitter' cultivars were preferred for snacks (Nweke, 1994). The use of raw roots from non-toxic cultivars as a thirst-quencher has been observed in South America, Africa and Asia (Jones, 1959; Fresco, 1986; Nweke & Bokanga, 1994). It should be noted that the distribution of mean glucoside levels in a collection of cassava cultivars from Nigeria and Cameroon was skewed towards low glucoside levels without any sign of a division into two groups (Bokanga, 1994). The reason may be that cultivars grown in these two countries do not fall into two distinct groups regarding cyanogenesis, or that the collection of cultivars was not representative of how commonly cultivars were grown, or that environmental effects in the agricultural research station influenced the distribution of glucoside levels.

Our earlier qualitative interviews in northern Malawi suggested the existence of a few cultivars in an intermediate group between 'cool' and 'bitter', but such cultivars may be rare as none were identified in the present study. Changed environmental conditions may have prompted the existence of the intermediate group through roots of some 'cool' cultivars becoming intermediate-tasting in harsh environments (Chiwona-Karltun *et al.*, 1998). Farmers may soon stop growing such cultivars since it is important that roots be stable in taste and safe to be eaten fresh. Several reports on cassava processing and utilisation in Africa do not mention that farmers perceive cassava as two different crops (Hahn, 1989). This may be due to the fact that many communities mainly grow either one or the other of the two types, or that the interview methods used were inadequate in documenting existing ethno-classifications (Rosling, 1995). Priority setting in cassava breeding that focused on yield and disease resistance (Hahn, 1989), may have overlooked that cultivars in many cassava-farming systems must fit either of the two groups to be adopted in most cassava-farming systems.

#### *Bitterness and food security*

The results confirm that cassava cyanogenesis is perceived as favourable for food security. Almost all the women stated that protection against theft and animal spoilage were very

important reasons for preferring to grow bitter cultivars as a staple crop. An interview study in six African countries concluded that farmers mainly prefer bitter cassava cultivars due to favourable flavour and texture of the end-products (Nweke & Bokanga, 1994). Enhanced food security was not reported as a reason for preferring to grow bitter cultivars. This reason may have been missed in many of the communities studied since the interviews may not have included the subtle probing questions needed to divulge sensitive issues like theft. A positive impact of cyanogenesis on food security was reported when adequate interview methods were used in Tanzania (Kapinga *et al.*, 1997). A study in Mozambique found that farmers preferred to grow bitter and toxic cassava cultivars due to attacks by monkeys since cyanogenesis reduced the crop loss (Cardoso *et al.*, 1998). Yet the authors suggest promotion of cultivars with low cyanogenic potential to prevent dietary cyanogen exposure. However, we assume that acyanogenic cultivars will not be accepted as the staple crop in food-insecure cassava-growing populations. The cassava root, being a storage organ, differs in several respects from the other staple crops from which the reproductive seeds or tubers are consumed. A major difference is that cassava roots can be stored in the ground for months or years, and another is that the roots are available during the dry period of the year when no other plant yields starchy roots and seeds. These characteristics of cassava contribute to its importance for food security and greatly increase the risk of theft of roots by vermin and humans. We find that food-insecure cassava farmers have good reasons to prefer bitter and toxic cultivars and the most efficient way to prevent dietary cyanogen exposure may be to promote adherence to traditional effective processing or the introduction of mechanical milling that appears to secure cyanogen removal by requiring that roots are well processed and dried (Banea-Mayambu *et al.*, 1998).

We are not aware of other reports that show bitterness of cassava roots deterring family members from unplanned harvesting and thereby empowering women to control household food security (Chiwona-Karltun *et al.*, 1998). This exemplifies the importance of gender roles in food production in developing countries. Other studies also show (Sperling &

Ntabomvura, 1994) that women farmers base selection of varieties on many more factors than yield and that taking women's needs and knowledge of indigenous farming systems into account is productive in the development of farming technology (Aarnink & Kingama, 1991; Ashby, 1994; Ilkharacan & Appelton, 1995). A few women in this study mentioned end-product quality as a major reason for preferring bitter cultivars. That better flour quality was attributed to bitter cultivars has been mentioned in earlier studies (Nweke & Bokanga, 1994), but nothing in this study suggests that cassava cyanogenesis *per se* contributes to end-product quality.

#### *Frequency of cassava consumption*

Of the women interviewed 81% reported consumption of *kondowole* during the last week and 51% reported consumption twice daily. Foods eaten in large amounts are estimated with fewer errors with food frequency questionnaire than those eaten in lesser quantities (Zulkifli & Yu, 1992; Willet, 1994). Furthermore, reported memory of food intake is strongly biased towards the time of data collection and the number of food items in the questionnaire (Joachim, 1997a, 1997b). This favours reliability of our results since we only covered consumption during the preceding week for 17 major food items. Yet, biases may have occurred in reported cassava consumption frequency as cassava in the past was neglected by Malawian authorities, and women may have perceived that investigators thought maize to be more socially desirable than cassava. During an informal discussion after completion of an interview, some women admitted to having adjusted their cassava consumption frequency downwards because they thought that the investigators wanted them to change their eating habits from cassava to maize. The consumption of snacks is commonly under-reported in food frequency studies (Zulkifli & Yu, 1992) and this might have affected our results on consumption of raw cassava roots as snacks between meals. This bias may only have resulted in lower than real reported frequency of cassava consumption (Herbert & Clemow, 1995; Kigutha, 1997). The frequency of *kondowole* consumption is higher in older women, heads of households and those that classify their families as being of lower wealth status. This may be due to cassava being

a cheaper source of energy, but also partly due to a more true reporting of cassava consumption frequency by this group (Worsley *et al.*, 1984). The dietary interviews undoubtedly showed that more than half of the women ate cassava daily as their main staple food, and previous answers showed that this staple food was made from bitter and toxic roots that were rendered safe by soaking and drying before milling into flour.

#### *Dietary cyanogen exposure*

Dietary cyanogen exposure occurs if cassava products contain residual amounts of linamarin or cyanohydrin, whereas cyanide rarely remains in significant amounts in consumed cassava products (Tylleskär *et al.*, 1992, White *et al.*, 1998). Linamarin may be broken down by glucosidases in the gut and the cyanohydrin formed may release cyanide that is absorbed into the blood stream where it is converted, mainly to thiocyanate. Linamarin may also be absorbed without causing cyanide exposure since it can be excreted intact in the urine (Mlingi *et al.*, 1992; Carlsson *et al.*, 1995). Cyanohydrins are expected to break down to cyanide at the high pH of the gut and the intake is assessed through urinary levels of thiocyanate. Nutritional status may aggravate the effect of cyanide exposure and decrease the representativeness of thiocyanate as a biomarker of cyanide exposure (Rosling, 1994). The relatively frequent consumption of fish probably provided the studied population with somewhat better protein status than has been the case in cassava-eating populations with very high dietary cyanide exposure as reflected by urinary thiocyanate above 500 µmol/l (Banea-Mayambu *et al.*, 1997). This supports the fact that the finding of low urinary thiocyanate in the present study really reflects a low exposure to cyanide. The very low urinary linamarin found in the present study indicates that almost all linamarin had been broken down or leached out of the roots during soaking. The mean urinary thiocyanate of 57 µmol/l in the daily *kondowole* consumers is a tenth of the levels observed in consumers of insufficiently processed bitter roots (Banea-Mayambu *et al.*, 1997) and in the same range as the levels in non-smoking Swedish subjects (Lundquist *et al.*, 1995). The slightly higher urinary thiocyanate in frequent *kondowole* consumers suggests

that this cassava product did result in a minor dietary cyanide exposure, which may be due to residual cyanohydrins in the flour. The studied population may, during other seasons in certain years, process and consume cassava in a different way that may expose them to cyanide. However, it is reasonable to conclude that daily consumption of flour from well-soaked and thoroughly dried roots of bitter cultivars only resulted in negligible cyanide exposure at the time of the present study when processing was strictly adhered to.

## Conclusion

Farmers in northern Malawi prefer to grow bitter cassava cultivars because cyanogenesis reduces the risk of theft and animal spoilage. The availability of water enables these farmers to process roots of bitter cultivars into a safe staple food. Promotion of safe cassava con-

sumption should be based on needs and possibilities in each farming and food system. In dry areas where soaking is not a viable option safety may be achieved by the promotion of cultivars with low cyanogenic potential, assuming that theft and animal spoilage are negligible problems. However, in many dry areas, theft and animal spoilage also constitute a threat to food security and farmers may opt to grow bitter cultivars even if this implies an increased risk for intoxication. Therefore, promotion of safe processing techniques appears to be necessary in almost all cassava-growing areas.

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# Paper III



# BITTER TASTE OF CASSAVA ROOTS STRONGLY CORRELATES WITH CYANOGENIC GLUCOSIDE LEVELS<sup>1</sup>

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# ABSTRACT

Cassava roots contain cyanogenic glucosides. Malawian farmers classify cassava cultivars into two groups based on the perceived danger of eating raw roots that they associate with bitterness. Roots from the 'bitter' and toxic cultivars are processed whereas roots of non-toxic "cool" cultivars can be eaten raw. We studied farmer's ability to predict cyanogenic glucoside levels of 492 roots of the ten most grown cultivars. Twenty-eight farmers predicted the taste of each cultivar and scored bitterness on a five-point scale. Cyanogenic glucosides were determined in one half of the root while a taste panel scored the other half.

Mean cyanogenic glucoside level in 132 roots from 'cool' cultivars was 29 mg HCN eq kg<sup>-1</sup> fresh weight (CI 25-33, range 1 - 123), and in 360 roots from 'bitter' cultivars 153 mg (CI 143 – 163, range 22 - 661). Farmers' distinction of 'cool' and 'bitter' cultivars predicts glucoside levels ( $r = 0.56$ ). Their taste scoring of each cultivar ( $r = 0.61$ ) and the tasting of the tip of the root ( $r = 0.65$ ) improved the predictions. The bitterness score of the taste panel and glucoside levels was strongly correlated ( $r = 0.82$ ) suggesting that cyanogenic glucosides confer the bitter taste.

Key words: *bitter taste; cassava; cyanogenic glucosides; farmers; perceptions; taste panel; sensory analysis; genetic; DNA; bitter; sweet; Malawi; Africa*

# INTRODUCTION

Several plant species are used as food or feed although they contain palatability reducing compounds or toxins<sup>1</sup>. Instead of excluding these plants from the diet, mankind has developed processing methods that reduce the levels of these compounds prior to consumption<sup>2-4</sup>. These compounds include the cyanogenic glucoside linamarin and lotustraulin found in cassava roots, an important staple crop in the tropics. When Amerindians several thousand years ago domesticated cassava cultivars that yield bitter and toxic roots, they also developed processing methods that reduced the cyanogen content and enabled them to eat the starchy root products from cassava as their main staple food<sup>5,6</sup>.

In many cassava farming and food systems, the cassava cultivars are classified into two main groups. In South America, where cassava originates and is the mainstay of the Amazonian Indians this classification also prevails. For instance, the term *kii* refers to "bitter" and *maksera* refers to "non-bitter" cassava<sup>7</sup>. In our previous studies from Malawi there is a similar classification *vyakubaba* for the "bitter" and *vyakuzizira* for the "cool or non-bitter"<sup>8</sup>. This classification is deemed critical in discerning the need for processing or not prior to consumption. Studies in Africa, have also shown that where cassava is predominantly used as a staple food the 'bitter' varieties dominate and where cassava is more fresh market oriented the 'non-bitter' varieties, also called "sweet", tend to dominate<sup>9</sup>.

The methods for processing cassava that have evolved in Africa have been adapted to suit the local preferences of the desired food products<sup>10,11</sup>. Roots from 'bitter' cultivars are preferred for attaining flour<sup>11</sup>. Similarly in South America, bitter cultivars are preferred for flour making<sup>5,7</sup>. The roots are processed either by direct sun-drying, by grating, fermenting and roasting in a pan over an open fire, by heap fermentation, or by soaking and fermentation, followed by sun drying. In contrast, roots from cultivars designated as 'sweet' or 'cool' are eaten raw as a hunger and thirst quencher, boiled, fried, roasted or processed to produce flour. In areas prone to food shortages the small-scale farmer in many parts of Africa has been observed to preferentially grow bitter cassava cultivars that yield toxic roots. The reason being that toxicity improves food security by conferring protection from theft, animal destruction, and unplanned harvesting by family members<sup>8,12-15</sup>.

Interview studies in Malawi with women farmers revealed that farmers use the bitter taste of cassava roots as an indicator of toxicity. They also considered it safe to eat processed products from bitter roots since their experience had shown that processing removed bitterness and that *kondowole*, the dumpling-like dish made from flour obtained from soaked and well-dried roots, did not induced any acute poisoning<sup>16</sup>.

The relationship between bitter taste and the level of cyanogenic glucosides of cassava roots has albeit been controversial. While some studies have shown that bitter taste in raw cassava roots correlates positively with levels of cyanogenic glucosides<sup>17-20</sup>, others have failed to find this association<sup>21,22</sup>. In northern Malawi, it is common practice for cassava farmers and consumers to predict toxicity of single raw roots by tasting the tip of the root parenchyma. To our knowledge, the ability of cassava farmers' to predict the levels of cyanogenic glucosides on the basis of local knowledge and taste has never been studied.

The first aim of the study was to elucidate to what degree cassava farmers could predict the potential toxicity of raw cassava roots using their local knowledge and practices. To achieve this aim we assessed the precision with which farmers could predict cyanogenic glucoside levels in raw roots by; (1) using their local classification of cultivars into two main groups called 'bitter' and 'cool', (2) their knowledge about the average degree of bitterness of roots from each cultivar, (3) their knowledge about the moderating effect of the environment in the fields on the bitterness of roots, and (4) their practice of tasting the root parenchyma of the tip of a single root. The second aim was to determine the correlation between taste and levels of cyanogenic glucosides in raw cassava roots using a trained sensory taste panel.

# METHODS

## Study Area

The study was conducted in Nkhata-Bay, the main cassava-consuming district in Malawi with well over 70% of the farmed land allocated to cassava<sup>8</sup>. The agricultural extension service divides the district into 53 sections. Two of these, Lweya and Mgodì, were selected for this study as being representative of the lakeshore zone. Each section comprised 8 blocks, each with about 100 farming households. One block from each section "Thowolo-B" and "Matyenda-1" were selected on the basis of being typical of the agricultural and social variation within each section. The study was approved by the Research Division and Extension Service of the Ministry of Agriculture, the District Health Commissioner in Nkhata-Bay, and by oral consent from the community leaders.

## Sampling

### *Selection of farmers*

13 households in "Thowolo-B" and 15 households in "Matyenda-1" were consecutively selected from the census list based on the following criteria. The cultivation of one or more of the ten most frequently grown cultivars, having at least two plants of each cultivar with roots ready for harvest in the same field, and the presence of the woman farmer previously interviewed to indicate the plants of each cultivar in her own field<sup>16</sup>. These 28 farmers made general predictions of the taste of the roots of each cultivar while being interviewed in their homes. They made a second prediction on the taste of the roots of each cultivar while in the field and finally they tasted the tip of the roots of the harvested cassava plants.

### *Farmers prediction and observation of taste and harvesting of roots*

In the present study, we sampled plants of the 10 cultivars that were reported grown by the highest proportion of households<sup>16</sup>. Out of these the farmers classified three as 'cool' and non-toxic and seven as 'bitter' and toxic cultivars. While in the field and prior to uprooting a pair of plants of each cultivar, the farmer was asked to predict their taste based on their experience of how roots of these cultivars usually taste in the particular field. In predicting taste they used a five-grade scale as follows: *very cool* (1), *cool* (2), *intermediate* (3), *bitter* (4) and *very bitter* (5). In this study, the taste scores *very bitter* and *very very bitter* were merged into one category as *very bitter*, a slightly different taste scoring scale from the previous study<sup>16</sup>. Thereafter, the farmer identified two plants as belonging to the specific cultivar and these plants were tagged before up rooting by the investigators.

### *Sample handling prior to analysis*

Independent persons in the investigating team carried out the harvesting and neither the farmer nor the principal investigator were present when the two biggest roots from each plant were packaged in labelled paper bags. The roots were given to the farmer one by one at the end of harvesting for them to cut a piece off the tip and to grade the taste on the same scale as indicated above. The roots were then repackaged and transported to Mkondezi Agricultural Research Station within six hours of harvest. Upon arrival they were peeled and washed by a trained group of assistants, using sharp stainless steel knives and potable tap water. The peeled roots were finally split longitudinally on plastic chopping boards and each half was put in labelled polythene bags and immediately used for the sensory and chemical analysis, respectively. A total of 492 roots from 246 plants stemming from 10 cultivars were collected for analysis. The number of plants and roots sampled from each cultivar are shown in table 1.

## Sensory Analysis

From the staff households at the agricultural research station, 30 healthy, non-smoking, non-habitual cassava consumers with at least eight years of education consented to participate in the taste panel. Following standard procedure the assessors were assessed on their ability to distinguish the lowest levels (0.6 mg caffeine/l water) of the bitter tasting compound caffeine. Four panel selection sessions were conducted and each prospective panellist was assessed twice. The 15 assessors that were able to identify the lowest concentrations of caffeine in solution from the blank sample were selected for the taste panel<sup>23,24</sup>. Roots from two cassava cultivars perceived by the farmers in Mkondezi area to be *very cool* ‘fyoka’ and *very bitter* ‘gomani’ in taste were used as standards for capturing the sensory space. These training sessions included discussions between the assessors and the food scientists. This was crucial for the distinction of the degree of bitterness along the five-grade scale.

The halved roots for the taste analysis were cut into 2-3 cm size pieces on plastic chopping boards and stored at room temperature in covered coded plastic bowls. The assessors sat separately in a room with good lighting and air conditioning with no facial view of one another. They were supplied with water for rinsing the mouth and a bucket for spitting the samples in. Coded root samples were served one at a time in multi-coloured plastic bowls in the afternoon starting from 14:00 hours. Each panel-list recorded the taste score of each root on a pre-coded sheet.

Assessors were served a standard lunch made from local dishes that excluded cassava at 12:00 noon during the 11 days of the study. Assessors followed the standard procedure i.e. rinsed their mouths with room-temperature water before and between samples. The root pieces were chewed but not swallowed and 48 - 50 samples were tasted each day. A mean taste score was calculated for each root from scores given by the 12-member panel. To avoid fatigue a 30 minute break was taken and assessors were served biscuits and orange squash halfway through the tasting session.

## Determination of glucoside levels

Cyanogenic glucoside analysis was done on each half of a root. The root was cut with a sharp stainless steel knife into approximately one cm sized cubes on plastic chopping boards. An amount of 49.5 -50.5 g was weighed into a plastic cup and mixed with 160 ml 0.1 M orthophosphoric acid using the method of Brimer<sup>25,26</sup>. Moisture content of each root was determined by oven drying of cubes until constant weight was attained.

## Quality control of assays

The reproducibility of the chemical analysis and the taste assessments was assessed by double determinations of 11 roots. Each of these roots was divided into four equal longitudinal portions to obtain double determinations for both mean taste score and the cyanogenic glucosides. The investigators conducting the taste panel and the chemical analysis were blinded for the doubling of these 11 samples. The eleven double estimations of mean taste score yielded a correlation coefficient of 0.95 and those of chemical determination of 0.99.

## Statistical analysis

Simple descriptive statistics, means, confidence interval, Anova and regression using SPSS version 9.0 were used to analyse the data

# RESULTS

## Farmers ability to use bitter taste as an assay for cyanogenic glucoside level

### *The local ethno-classification into 'bitter' and 'cool' cultivars*

Table 1 shows a considerable gap between the mean glucoside levels of the seven 'bitter' and the three 'cool' cultivars, respectively. The mean cyanogenic glucoside level for all the 132 roots from the three 'cool' cultivars was 29 mg HCN equivalents/ kg fresh weight (CI = 25-33; range 1 - 123). The 360 roots from the 'bitter' cultivars had a mean glucoside level of 153 mg HCN equivalents/ kg fresh weight (CI = 143 - 163; range 22 - 661). Figure 1 shows that there is a minor overlap in cyanogenic glucoside levels between the roots from cultivars designated as 'cool' and as 'bitter' respectively.

### *General knowledge about the character of each cultivar*

Column three in table 1 shows the mean score of the farmer's prediction of taste while at home for each cultivar. While at home farmers predicted 'nyamakozo' to be the most bitter tasting cultivar and it had the highest mean cyanogenic glucoside level. Within the bitter cultivars, farmers further predicted the cultivars 'depweti', 'nyaharawa' and 'koloweki' to be less bitter than the other 'bitter' cultivars. The roots of these three cultivars had lower cyanogenic glucoside levels than that of the other 'bitter' cultivars. For the seven 'bitter' cultivars there was a statistically significant ( $p<0.05$ ) positive correlation ( $r = 0.82$ ) between the mean taste score for predicted bitterness and the mean glucoside levels of the roots. With log transformation of cyanogenic glucoside levels the r-value increased to 0.89 ( $p<0.01$ ).

### *Farmers' ability to predict environmental effect*

Table 1 shows the mean taste scores of the farmer's prediction for each cultivar when trying to consider the modifying effect of the environment on each field. The general predictions for the taste of each cultivars made in their homes gave higher mean taste scores than those made in the fields and the correlation between glucoside level and predicted taste was higher when made in the home. There is a positive ( $r = 0.46$ ) but not statistically significant correlation between the mean predicted taste scores made in the fields and mean cyanogenic glucoside levels (whether log transformed or not) for the seven 'bitter' cultivars. Table 2 shows the mean cyanogenic glucoside levels for roots of each cultivar when desegregated by predicted taste score given by the farmers for each cultivar in the environment of a given field. The table shows that farmers predicted taste score was statistically significantly associated to higher mean glucoside levels for only three of the seven 'bitter' cultivars.

### *Farmers' tasting of the tip of the cassava root*

Table 3 shows the mean glucoside levels for each cultivar desegregated by the taste score by the farmers for each individual root after tasting the tip of the root. The mean cyanogenic glucoside level for nine out of the ten cultivars is statistically associated with farmers' assessment of taste. There was a strong positive correlation ( $r = 0.65$ ) between the glucoside level and the taste score given by farmers for all the 492 roots. With logarithmic transformation of the glucoside levels the r-value increased to 0.73. There was also a strong positive correlation ( $r=0.83$ ) when comparing the mean taste score for each cultivar and the mean glucoside level from farmers tasting of the root tip and mean glucoside levels (linear and logarithmic) for each of the 7 'bitter' cultivars (table 1).

## Correlation between taste and cyanogenic glucoside level

Figure 1 shows a high correlation between the cyanogenic glucoside levels of the 492 roots on logarithmic scale ( $r = 0.87$ ) and the mean taste score from the sensory panel for each root. The  $r$ -value without logarithmic transformation is 0.82. The mean taste score and glucoside level overlap between roots from 'bitter' and 'cool' cultivars. There is a large dispersion of cyanogenic glucoside levels occurring between the mean taste scores of 4.5 and 5.0, i.e. in roots observed as being very bitter in taste by most panellists. Table 4 shows the mean glucoside levels for the roots of each cultivar desegregated by the taste scoring of the panellists. The mean taste scores of the panel have been categorised into the five taste categories. In contrast to the results from farmers tasting in table 3 the results in table 4 shows that there is a statistically significant association between taste and glucoside levels for all the ten cultivars.

The summary of correlation co-efficient analysis in table 5 showed that the farmers' tasting of the tip of the roots could predict cyanogenic glucoside levels ( $r = 0.65$ ). However, the taste scoring of the longitudinal half of each root by the taste panellists was more robust in predicting cyanogenic glucosides  $r = 0.82$  (linear).

## DISCUSSION

### Farmers' ability to use taste in predicting toxicity

The mean cyanogenic glucoside levels of roots for each cultivar fell into two groups that coincided with farmers' ethno-classification of cultivars into two groups (table 1). All 7 'bitter' cultivars have much higher mean glucoside levels than the 3 'cool' cultivars. Earlier interviews<sup>16</sup> revealed that farmers regarded 'bitter' and 'cool' cassava cultivars as being two different food crops having different functions in the food system. 'Bitter' cultivars were grown for processing into flour and farmers preferred these roots to be bitter and dangerous as a protective measure for their staple crop. In contrast, the roots from 'cool' cultivars were largely used as snacks without prior processing and they were stated to be non-bitter and safe to eat raw. The findings of different mean cyanogenic glucoside levels in the two groups strongly confirms that farmers ethno-classification into two groups of cultivars is based on distinctly different toxic potential of the roots. This is in agreement with findings among Indians in South America<sup>7</sup> that also consider cassava cultivars to belong to two different groups. It is also reported from other areas that farmers grow bitter and toxic cultivars as a staple crop and the non-bitter and non-toxic, mostly referred to as sweet cultivars, to be eaten without the processing needed to remove toxicity<sup>11</sup>. It has been assumed that the distinction of cultivars into two groups corresponds to a several-fold difference in the levels of cyanogenic glucosides<sup>7</sup>. To our knowledge, this is the first study that confirms this difference within a cassava farming system.

The farmers' knowledge of the degree of bitterness of roots from each cultivar were measured by the mean taste score for each cultivar as predicted by each farmer when interviewed in their home. Interview studies in the same area revealed that farmers equated the degree of bitterness of roots with their potential toxicity if eaten raw<sup>16</sup>. In this study we found a significant and positive correlation between the mean taste score and the mean glucoside level of the seven 'bitter' cultivars. This strongly indicates that farmer's local knowledge enables them to predict the degree of potential toxicity of each 'bitter' cultivar. The range of variation of mean glucoside levels was very narrow for the three 'cool' cultivars and farmers also predicted them to have almost the same taste. The local ethno-classification thus conveys farmer's knowledge regarding taste and thus predicted potential toxicity in two ways. First by the distinction of the two groups of cultivars, 'bitter' and 'cool', and secondly by specific knowledge linked to each of the named cultivars within the group of 'bitter' cultivars.

The results suggest that farmers are not capable of predicting the moderating environmental effect on the glucoside levels of cassava roots. Only for three of the seven 'bitter' cultivars could it be shown

that farmers in predicting the taste of roots in a given field could predict the environmentally induced variation in glucoside levels. The correlation between glucoside levels and taste predictions for each of the ten cultivars was actually stronger when made in general terms for the cultivars compared to when made for plants grown in a specific field. The reason for this may be that the range of environmental variation was small in this study and that the farmers felt more pressured to have the 'right' prediction when interviewed in the field than in their homes. Another explanation is that the environmental effect on glucoside levels is simply unpredictable.

The taste scores given by farmers' tasting the tip of the root was strongly associated with the content of cyanogenic glucosides in the whole roots. The only exception is the cultivar 'nyaharawa' in which farmers scoring of the taste of the tip is not statistically significantly associated with glucoside levels. However, the mean taste score of the sensory panel and the glucoside levels are statistically significantly strongly associated even for 'nyaharawa' (table 4). The reason for this discrepancy may be that the glucosides are more irregularly distributed along the longitudinal axis of the root in 'nyaharawa'. Farmers assessed the taste of the tip of the root as was the usual practice, therefore they were not able to predict the glucoside levels as well as the sensory panel that collectively tasted a representative sample of a longitudinal half of each root. Nevertheless, our results show that farmers have a high ability to predict the potential toxicity of an individual root by just tasting the tip of the parenchyma of the root.

In table 3 the mean levels of cyanogenic glucosides are higher for roots of 'bitter' cultivars when scored to have the same taste score as roots of 'cool' cultivars. Two different effects may explain this. First that the roots from 'cool' cultivars that were scored to have *very cool* or *cool* taste mainly fell in the lower part of that category. In contrast the roots from 'bitter' cultivars that were scored into the same taste category tended to be in the upper part of the category. This effect probably explains most of the differences in glucoside levels between roots being ranked into the same taste category by farmers as well as the sensory panel. Another possibility is that the taste scores given by farmers for some roots from 'cool' cultivars were exaggerated. The tasting of the cassava roots by the farmers was designed to be a blind study for the farmers and the main investigator administering the root tasting. However, we discovered that the farmers in most cases were able to identify the roots as belonging to a certain cultivar just by looking at the outer skin morphology, therefore a certain influence from their experience about each cultivar cannot be excluded. When the farmers experienced some bitter taste in roots from the 'cool' cultivars their statements may reflect that the taste was bitter for being roots from 'nyachikundi' rather, than objectively using the same scale as for roots of bitter cultivars. The latter explanation is supported by the fact the 'nyachikundi' roots considered to be very bitter in taste by farmers were considered to be only intermediate or bitter by the sensory panel.

The correlation co-efficient analysis in table 5 showed that farmers with acceptable precision can predict toxicity of cassava roots not only prior to tasting  $r = 0.59$ , but that their prediction improved, if they taste the tip of individual roots  $r = 0.65$ .

## Correlation between taste and cyanogenic glucoside level

The results of the chemical analysis and the taste panel assessment (Fig 1) are consistent with the farmer's perceptions that taste predicts toxicity in raw cassava roots<sup>8,16</sup>. As seen in fig 1, the largest dispersion of cyanogenic glucoside levels occurs between the mean taste scores of 4.5 and 5.0, that is the roots that most taste panellists observed as being very bitter in taste. The explanation for this may be that the five-score scale for taste scoring was too narrow and/or that the very bitter roots may have saturated the bitter tasting taste buds. In spite of this large dispersion at the upper-end, the overall correlation between taste and level of cyanogenic glucosides in the roots is strong.

Statements referring to bitter taste of cassava root parenchyma being equated with high cyanogenic glucoside content are numerous in review literature<sup>4,5,10,12,28-30</sup> but the methodology is only partially described. Published studies specifically on this relationship are few and conflicting. Two early studies<sup>17,18</sup> conducted a taste panel on raw cassava roots and measured cyanogens. They demonstrated

a strong correlation between bitter flavour of root parenchyma and the yield of hydrocyanic acid from roots and concluded that taste could be used in the selection of new varieties for human use. The authors attributed the bitter taste of cassava roots to be mostly due to the content of cyanogenic glucosides. A possible reason for why it has been problematic to ascertain the relationship between cyanogenic glucoside levels and bitter taste may be due to the wide variation in cyanogenic glucoside levels in the different parts of one cassava root<sup>27</sup>. In the present study, this variation was circumvented by longitudinally cutting the root into two equal halves. Each of the root halves was further divided into pieces and samples for tasting and chemical analysis were randomly drawn from the whole mixture.

In a study from India, roots from 33 varieties were analysed for HCN yield and taste<sup>21</sup> but no further details of the taste panel composition or taste procedure are given. On the basis of taste, roots were classified into four categories very bitter, bitter, non-bitter and sweet. Out of the 33 varieties: 3 were very bitter, 11 bitter, 14 not bitter and 5 sweet. The very bitter varieties had HCN values that ranged from very bitter 275 - 490, bitter 86 - 406, non-bitter 24 - 181 and the sweet 35 - 130 mg/kg in the flesh of tubers. Interestingly, the authors on this basis conclude that the amount of HCN alone does not seem to be the deciding factor for bitterness.

A similar study by Pereira *et al.*,<sup>22</sup> three cultivars were defined as having low cyanide yield and the other three as being high in cyanide yield. In contrast to their earlier findings<sup>17,18</sup>, they found no association between HCN yield and the taste in this later study.

Sundaresan *et al.*,<sup>19</sup> compared the taste of roots from 38 cassava varieties and the cyanogenic glucoside content, but the paper did not state whether the taste analysis and the chemical analysis were done on representative samples of the same root. Roots were categorised by a taste panel of five persons using a scale with three taste levels, however the detailed methods were not presented. Non-bitter roots had cyanogenic glucoside levels of 27.5 – 77.5, bitter roots 100 – 180 and very bitter roots 320 – 1100 mg HCN eq/g roots, respectively. The finding support that the cyanogenic glucosides convey the bitter taste nevertheless, however, it is surprising that there was no overlap of the cyanogenic glucoside levels between the three categories of taste.

Our finding of a strong correlation between glucoside level and bitterness is consistent with the majority of the earlier studies. There are two possible explanations for this strong correlation between bitter taste and the level of cyanogenic glucosides. The first is that the cyanogenic glucosides account for most of the bitter taste in the root parenchyma. This is supported by an experimental study by King and Bradbury<sup>20</sup>. Although they found several bitter tasting compounds in the peel, they concluded that linamarin is the sole contributor of the bitter taste in the parenchyma of cassava roots. The second possibility is that the levels of one or several bitter tasting compounds are strongly genetically coupled with the cyanogenic glucoside levels, and the cyanogenic glucosides do not have a bitter taste. This is supported by an unpublished observation<sup>28</sup> on roots from one cultivar known in Benin Republic as ‘manioc mortel’ (deadly cassava) that had a sweet taste but cyanogenic glucoside levels of 350 mg HCN eq kg<sup>-1</sup>. In our present study only one out of 492 roots had high glucoside levels (239 mg HCN eq kg<sup>-1</sup>) in spite of being graded as cool in taste (taste score 2.2). It is interesting to note that this root (Fig 1) came from a genetically atypical ‘nyaharawa’ cultivar and this could possibly be the explanation (Mkumbira J *et al.*, unpublished). Since the other root from the same plant did not deviate from the general trend, i.e. bitter tasting predicting cyanogenic glucoside levels, a more probable explanation may be that it is an artefact that occurred during the experiment or analysis. Other substances in the parenchyma, evidently, in addition to the cyanogenic glucosides might contribute to the bitter taste<sup>17,20,21</sup>. Nevertheless, the strong correlation ( $r = 0.87$ ) found between the cyanogenic glucoside levels of cassava root parenchyma and bitter taste, using a robust sampling and sensory analysis methodology suggest that linamarin may be responsible for the bitter taste of cassava roots. This remains to be confirmed with carefully designed experimental studies on the taste of the pure forms of cyanogenic glucosides, and on the taste modifying factors in the rare cassava genotypes with high cyanogenic glucoside level and no bitter taste.

# CONCLUSION

We conclude that farmers in this study are able to predict toxicity of the roots of their local cassava cultivars on the basis of bitter taste with high precision. Prediction of bitterness is strengthened when the farmers actually taste the tip of the raw root. We find it most probable, although not yet fully proven that the bitter taste in cassava roots is conveyed by cyanogenic glucosides, mainly linamarin.

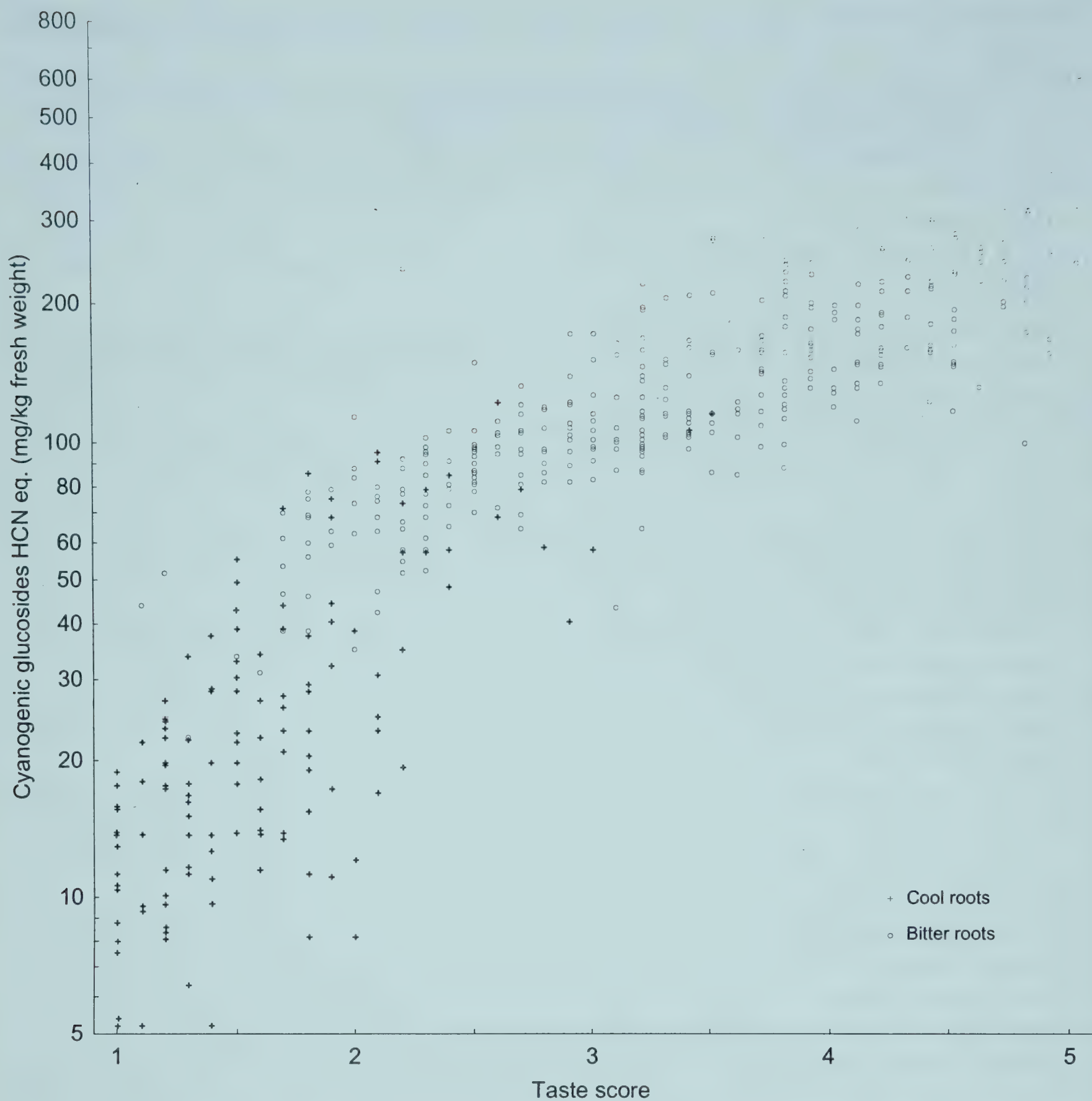
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**Figure 1. Correlation between the levels of cyanogenic glucosides (logarithmic) and the mean taste score as determined by the sensory panel for each of the 492 roots**

**Table 1. Mean±SEM taste scores and cyanogenic glucoside levels as mg HCN eq kg<sup>-1</sup> fresh weight for roots of each of the ten cultivars**

Cultivar	General assessment of each cultivar while at home*		Assessment of plants of each cultivar in the field prior to harvesting		Assessment of roots as tasted by farmers, by taste panelists and chemical analysis			
Type and name	No. of farmers	Mean taste score	No. of plants	Mean taste score	No. of roots	Farmers taste score	Panelists taste score	Cyanogenic glucosides
<b>‘Bitter’</b>								
Nyamakozo	10	4.9±0.1	24	4.3±0.1	48	4.2±0.1	4.3±0.1	242±2
Gomani	21	4.6±0.1	28	4.1±0.1	56	3.3±0.1	3.7±0.1	161±1
NyaNkhata	8	4.8±0.2	26	4.8±0.1	52	3.7±0.2	3.4±0.1	160±1
Ngwenyani	7	4.8±0.2	26	4.5±0.1	52	3.9±0.2	3.4±0.1	158±1
Depweti	22	4.3±0.2	26	3.2±0.2	52	3.4±0.2	3.1±0.1	120±7
NyaHarawa	9	4.1±0.2	24	4.1±0.1	48	3.1±0.2	2.9±0.1	117±9
Koloweki	12	4.1±0.2	26	3.5±0.1	52	3.2±0.2	3.1±0.1	114±7
<b>‘Cool’</b>								
NyaChikundi	7	1.3 ±0.3	22	1.6±0.1	44	1.6±0.2	1.8±0.1	31±4
Chimpuno	8	1.3 ±0.3	20	1.2±0.1	40	1.2±0.1	1.6±0.1	30±4
Mbundumali	20	1.0 ±0.0	24	1.3±0.1	48	1.1±0.1	1.5±0.1	25±3

\*Mean of general taste scoring for each cultivar obtained from the same woman in an earlier field survey  
(Chiwona-Karltun et al., 1998)

**Table 2. The mean cyanogenic glucoside level\* for roots from each cultivar grouped according to predicted taste scoring by farmers while standing at the edge of the field**

Cultivar	Number of roots	Taste score category of plants as predicted by farmers prior to harvesting					Anova sig test p-value
Type and Name	n = 492	very cool	cool	intermediate	bitter	very bitter	
<b>‘Bitter’</b>							
Nyamakozo	48			195±19	170±23	279±29	.033
		(0)	(0)	(16)	(4)	(28)	
Gomani	56			152±22	140±16	173±15	.393
		(0)	(0)	(20)	(8)	(28)	
Nyankhata	52				120±9	172±15	.065
		(0)	(0)	(0)	(12)	(140)	
Ngwenyani	52			125±20	120±31	174±18	.120
		(0)	(0)	(8)	(8)	(136)	
Depweti	52		111±8	107±12	147±23	145±17	.036
		(0)	(16)	(20)	(4)	(12)	
Nyaharawa	48		109±9	115±14	120±13	117±17	.834
		(0)	(4)	(8)	(16)	(20)	
Koloweki	52		66±11	102±6	127±10		.008
		(0)	(16)	(16)	(32)	(0)	
<b>‘Cool’</b>							
Nyachikundi	44	23±3	30±5	89±13			<.001
		(24)	(16)	(4)	(0)	(0)	
Chimpuno	40	31±5	28±9				.795
		(32)	(8)	(0)	(0)	(0)	
Mbundumali	48	23±3	30±4				.265
		(36)	(12)	(0)	(0)	(0)	

\*The mean±SEM glucoside level in mg HCN eq. kg<sup>-1</sup> fresh weight. Number of roots in each taste score category in parenthesis.

**Table 3. The mean cyanogenic glucoside level\* for roots from each cultivar grouped according to scoring of *observed taste by farmers after tasting the tip of each root***

Cultivar	Number of roots	Taste score category of plants as tasted by farmers					Anova sig Test p-value
Type and Name	n = 492	very cool	cool	intermediate	bitter	very bitter	
<b>‘Bitter’</b>							
Nyamakozo	48		136	181±17	211±28	294±32	0.004
		(0)	(1)	(15)	(8)	(24)	
Gomani	56	60±12	100±11	133±11	199±21	253±31	< 0.001
		(3)	(8)	(21)	(15)	(9)	
Nyankhata	52	130±19	102±13	122±16	170±34	205±19	0.001
		(2)	(7)	(14)	(9)	(20)	
Ngwenyani	52		116±18	127±16	123±12	214±29	0.005
		(0)	(7)	(13)	(12)	(20)	
Depweti	52	75±12	81±7	108±9	153±20	146±11	< 0.001
		(2)	(10)	(16)	(11)	(13)	
Nyaharawa	48	117	127±15	133±17	73±12	107±16	0.151
		(1)	(15)	(17)	(8)	(7)	
Koloweki	52	66±11	87±7	113±11	112±13	170±15	0.001
		(4)	(10)	(19)	(10)	(9)	
<b>‘Cool’</b>							
Nyachikundi	44	20±2	54±12	69	11	81±14	< 0.001
		(31)	(8)	(1)	(1)	(3)	
Chimpuno	40	24±4	51±13				0.009
		(31)	(9)	(0)	(0)	(0)	
Mbundumali	48	23±2	58±13				0.001
		(45)	(3)	(0)	(0)	(0)	

\*The mean±SEM glucoside level in mg HCN eq. kg<sup>-1</sup> fresh weight. Number of roots in each taste score category in parenthesis.

**Table 4. \*The mean cyanogenic glucoside level for roots from each cultivar grouped according to taste scoring of roots by taste panellists**

Cultivar	Number of roots	Taste category scores of plants as scored by taste panellists					
Type and Name	n = 492	very cool	cool	intermediate	bitter	very bitter	Anova sig Test p-value
<b>‘Bitter’</b>							
Nyamakozo	48			143±23	185±13	325±33	< 0.001
		(0)	(0)	(5)	(22)	(21)	
Gomani	56		72±7	111±8	181±16	249±23	< 0.001
		(0)	(8)	(15)	(21)	(12)	
Nyankhata	52	23	69±12	123±10	181±17	268±26	< 0.001
		(1)	(6)	(18)	(18)	(9)	
Ngwenyani	52		74±5	111±5	190±14	333±25	< 0.001
		(0)	(8)	(20)	(18)	(6)	
Depweti	52	52	77±6	112±6	158±11	193±34	< 0.001
		(1)	(13)	(21)	(12)	(5)	
Nyaharawa	48		75±12	107±5	203±16		< 0.001
		(0)	(15)	(23)	(10)	(0)	
Koloweki	52	44	69±5	100±5	158±10	193±34	< 0.001
		(1)	(12)	(20)	(17)	(2)	
<b>‘Cool’</b>							
Nyachikundi	44	16±2	29±4	78±10	115		< 0.001
		(13)	(26)	(4)	(1)	(0)	
Chimpuno	40	16±2	37±6	123			< 0.001
		(17)	(22)	(1)	(0)	(0)	
Mbundumali	48	14±1	39±5	50±9			< 0.001
		(27)	(19)	(2)	(0)	(0)	

\*The mean±SEM glucoside level (mg HCN eq. kg<sup>-1</sup> fresh weight). Number of roots in each taste score category in parenthesis.

**Table 5. Correlation between predictions and assessment of taste and cyanogenic glucoside levels in roots**

	Correlation co-efficient r
Ethno-classification into ‘bitter’ or ‘cool’ cultivars	0.56
Farmers' general predicted taste for each cultivar at home	0.61
Farmers' specific predicted taste of each cultivar in each field	0.59
Observed taste of the tip of the root by farmers	0.65
Observed taste of longitudinal half root by taste panel	0.82



# Paper IV



# CLASSIFICATION OF CASSAVA INTO “BITTER” AND “COOL” IN MALAWI: FROM FARMERS’ PERCEPTION TO DNA CHARACTERISATION

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# SUMMARY

Cassava roots, a major food in Africa, contain cyanogenic glucosides that may cause toxic effects. Malawian women farmers considered fields of seemingly similar cassava plants to be mixes of both 'cool' and 'bitter' cultivars. They regard roots from 'cool' cultivars as non-toxic. Roots of 'bitter' were considered to require extensive traditional processing done by women to be safe for consumption. But curiously, these women farmers preferred 'bitter' cultivars since toxicity confers protection against theft that was a serious threat to the food security of their families. We studied how well these farmers comprehend cassava genetics in dealing with cyanogenesis in this complex system. Using molecular markers we show that most plants farmers identified as belonging to a particular named cultivar had a typical genotype of that cultivar. Farmers' ethno-classification into 'cool' and 'bitter' cultivars corresponded to a genetic sub-division of the typical genotypes of the most common cultivars with four-fold higher cyanogenic glucoside levels in the bitter cultivars. Examining morphology, farmers distinguished genotypes better than did the investigators when using a standard botanical key. Undoubtedly, these women farmers grasp sufficient of the genetic diversity of cassava with regard to cyanogenesis to simultaneously benefit from it and avoid its dangers. Consequently, acyanogenic cassava is not a priority to these farmers. Advances in molecular genetics can help improve food supply in Africa by rapid micropropagation, marker assisted breeding and transgenics, but can also help to elucidate tropical small-scale farmers' needs and skills.

*Key words:* cassava cultivars, cyanogenic glucosides, DNA characterisation, farmers' perception, genotype, *Manihot esculenta*

# INTRODUCTION

The starchy roots of cassava, *Manihot esculenta* Crantz, have become the most important source of dietary energy in Sub-Saharan Africa (FAO 2000; Scott et al. 2000). This is due to the high and stable yield (Akoroda 1995), especially in areas with arable land shortage and declining soil fertility (Romanoff & Lynam 1992). Other advantages of cassava are its flexible planting and harvesting time, its suitability for inter-cropping and that it is vegetatively propagated. Most small-scale cassava farmers grow a number of cultivars, each with locally preferred qualities such as good taste, early maturing or good processing characteristics (Salick et al. 1997; Chiwona-Karltun et al. 2000). African small-scale farmers mainly acquire new cultivars from their neighbours, during travels or by collecting seedlings of sexually propagated cassava (*volunteers*) found in fields left in fallow for several years (Chiwona-Karltun et al. 1998). The performance of a cultivar within the local environment and farming system determines whether it will be adopted, and continue to be cultivated. There is evidence from several parts of Africa that few cassava cultivars originate from breeding programmes (Spencer 1994; Nweke et al. 1994; Chiwona-Karltun et al. 1998).

An earlier study in one area of northern Malawi showed that farmers have descriptive names for up to 50 cultivars just in one village (Chiwona-Karltun et al. 2000). The number of plants grown of each cultivar differs considerably and it changes over time. Cultivars may be ‘killed’ when their yield becomes unsatisfactory. The local cultivar names denote phenotypic attributes, place of origin, the person that introduced it or that it originates from a volunteer seedling (Chiwona-Karltun et al. 1998). As in many other areas the Malawian small-scale farmers grow a mix of cultivars with seemingly similar plants and claim that some cultivars yield roots that can be eaten raw whereas others yield “bitter” roots that must be subjected to elaborate processing before consumption (Dufour 1988; Chiwona-Karltun et al. 2000). “Bitter” taste of the roots is associated with a high risk of poisoning if not processed prior to consumption.

Previous studies in Malawi revealed an ethno-classification of cassava cultivars into two groups based on whether the roots could be eaten raw without prior processing or if they needed to be processed before consumption. In the local language these groups were called “cool” and “bitter” (Chiwona-Karltun et al. 1998; Chiwona-Karltun et al. 2000). A similar division of cassava cultivars into two groups, mostly referred to in English as “sweet” and “bitter”, has been reported from many areas (Nordenskiöld 1924; Schery 1947; Sauer 1963; Ugent et al. 1986; Dufour 1988; Nweke 1995). Exclusive cultivation of “sweet” cultivars is mostly found where cassava only comprises a small part of a diverse crop system (Cousins 1903; Nordenskiöld 1924; Renvoize 1972; Allem 1994; Dufour 1995). In areas where cassava is the main crop the “bitter” cassava cultivars dominate and “sweet” cultivars are grown in less amount or not at all (Purseglove 1968; Lathrap 1973; Dufour 1993; Dufour 1995; Nweke et al. 1994; Chiwona-Karltun et al. 2000; Nweke et al. 2001). The division of cassava cultivars into “sweet” and “bitter” has not been found to correlate with phenotypic features (Rogers & Appan 1973; Rogers & Fleming 1973) and to our knowledge the character of this division has not been studied in Africa using modern molecular genetic markers.

Advances in molecular genetics lend promise in the development of acyanogenic cassava. This would undoubtedly improve the understanding of the biological role of cyanogenesis in cassava. However, caution is needed before considering acyanogenesis as a means of preventing possible poisoning from cassava consumption. The reason being that the small-scale farmers at risk of dietary cyanide exposure from cassava seems to prefer bitter cultivars since the bitter taste and toxicity of the roots confer protection against theft and attacks by vermins. We therefore found it prudent to also apply the advances in molecular plant genetics to elucidate how small scale farmers presently understand, handle or fail to handle the genetic diversity of cassava with respect to cyanogenesis.

This study investigated the local ethno-classification of cassava using Short Sequence Repeat (SSR) markers on material obtained by a survey in northern Malawi. The first aim was to verify to what degree each of the 10 most grown cultivars constituted a single or a mixture of genotypes. The second

aim was to determine the accuracy with which farmers are able to identify plants as belonging to the named cultivars. The third aim was to ascertain to what degree the classification of “bitter” or “cool” cultivars comprised two different genetic pools. In this paper the term cultivar denotes the different types of cassava that are recognised within a local farming system by a specific local name and the term genotype implies a specific allele pattern found in eight SSR loci.

## METHODS

### Study Area

The study was conducted in Nkhata-Bay district with a predominantly rural population of approximately 165,000 (Malawi 2000). It is situated along the shore of lake Malawi in the northern part of the country. Cassava is the major staple crop, in this district, with about 70% of the farmed land allocated to cassava (Musukwa & Pelletier 1990). The district comprises four agro-ecological zones: the islands of Likoma and Chizumlo, the lakeshore zone between 475 – 600 m above sea level, the escarpment between 600 – 900 m and the less densely populated mountainous plateau zone above 900 m. The district is divided into 53 agricultural extension sections. Each section is further divided into 8 blocks each with about 100 farming households. An agricultural extension worker, known as a Field Assistant (FA) is responsible for each section. Two adjacent sections, Lweya and Mgodì, were selected for this study on the basis of being regarded as representative for the lakeshore zone (Chiwona-Karlton et al. 1998). The dominant ethnic groups in both sections were the Tonga. One block from each section “Thowolo-B”, and “Matyenda-1” were selected on the basis of being “typical” of the agricultural and social variation within each section. “Thowolo-B”, and “Matyenda-1” comprised 98 and 102 households, respectively and they were located about 3 km apart (Chiwona-Karlton et al. 2000).

The farming communities in both blocks have a long tradition of growing cassava and many of the name given cultivars have been grown for more than fifty years (Berry & Petty 1992; Chiwona-Karlton et al. 1998). Cassava roots from “bitter” cultivars are preferentially soaked, fermented, dried and pounded into flour. The flour is used to make the staple food *kondowole*, a dumpling-like dish that is eaten with the fingers together with a sauce. The farmers actively select cultivars through informal exchange of stem cuttings or from collecting *volunteer* plants from re-grown fallow fields. Cassava is mainly grown by women in small fields as a mix of plants of up to 15 cultivars that are largely maintained by vegetative propagation of stem cuttings. Since everyone in the area is essentially a cassava farmer and since there are hardly any market opportunities, the harvesting of cassava for household consumption is done on a piecemeal basis throughout the year. Most of the planting is done in direct relation to the piecemeal harvesting. This results in fields with a mix of plants of different cultivars and at different ages.

The study was approved by the Departments of Agricultural Research and Extension Service of the Ministry of Agriculture, the District Health Commissioner in Nkhata-Bay, and by oral consent from the community leaders and farmers.

### *Main survey in August/September 1996*

A study in the same study area in July 1996 started with a house-to-house survey of all the 200 households. The 176 eligible women being in charge of cooking and farming in these households were interviewed and reported growing a total of 45 name-given cassava cultivars (Chiwona-Karlton et al. 2000). Plants of the 10 cultivars reportedly grown by the largest proportion were sampled during the main survey in August and September 1996. Three cultivars were designated as “cool” (c) and seven as “bitter” (b). Out of the 176 women farmers interviewed 92 reported growing ‘mbundumali’ (c), 31 ‘chimpuno’ (c), 31 ‘nyachikundi’ (c), 122 ‘gomani’ (b), 113 ‘depwete’ (b), 76 ‘koloweki’ (b), 52 ‘nyaharawa’ (b), 37 ‘nyankhata’ (b), 27 ‘ng’wenyani’, and 23 (b) ‘nyamakozo’ (b).

From the household census list compiled in July 1996 (Chiwona-Karltun et al. 2000) we consecutively sampled 30 households for this study. One household from “Matyenda 1” was excluded because the woman interviewed in July was not available and one from “Thowolo B” due to later failure of extracting DNA from the plants collected. Thus a total of 28 farmers from these households were finally included, 13 in “Thowolo-B” and 15 in “Matyenda-1”. The criteria for inclusion was the presence of the woman farmer previously interviewed to indicate the plants of each cultivar in her own field. In addition the cultivation of one or more of the ten most frequently grown cultivars, and having at least two plants of each cultivar in the same field with roots ready for harvest.

Identification of plants by farmer was done under observation (LC-K). Each of the 28 farmers walked through their own fields to identify pairs of plants that they claimed belonged to one of the ten cultivars. Farmers were asked to only identify plants regarded as being ready for harvest. They did this by carefully examining the various plants including parts of the roots that could be observed by unearthing with their hands. The number of cultivars with plants ready for harvest that could be identified by each farmer ranged from one to eight and the survey continued until about 20 plants of each of the 10 cultivars had been collected. All 246 plants collected were independently identified by the farmers, immediately labelled (HR) and within minutes examined and collected (JM, NM) by the investigators.

Morphological characterisation of above and below ground parts of each plant was conducted (JM and NMM) using a modified cassava morphological descriptor (Nweke et al. 1994). The eleven morphological characters that were assessed are: shoot pubescence, shoot colour, leaf colour, leaf shape, petiole colour, leaf lamina colour, mature stem colour, root neck length, outer root skin colour, inner root skin colour and root pulp colour. The categories of each character are shown in footnote in table 1.

Two mature roots from each plant were collected for chemical analysis immediately after the morphological characterisation. The roots were transported to Mkondezi Agricultural research station chemical laboratory (approximately 70 km north of the study area) in the early hours of the afternoon on each day. In the laboratory, the roots were peeled, washed and split longitudinally. One longitudinal half was cut (with a sharp stainless steel knife) into approximately one cm sized cubes on plastic chopping boards. An amount of 49.5 -50.5 g was weighed into a plastic cup and mixed with 160 ml 0.1 M orthophosphoric acid and homogenised. Cyanogenic glucoside levels were determined using the methods of Brimer et al. (1997) and Saka et al. (1998). The mean value for the two roots of each plant was calculated.

Labelled stem cuttings were collected and planted in a nursery at Mkondezi Agricultural Research Station. At the end of the four week survey and with due permission from the Ministry of Agriculture in Malawi, the cuttings were transported to Sweden and re-planted in a greenhouse at the Swedish University of Agricultural Sciences, Uppsala. Following leaf development after about one month, DNA was extracted from two fresh unexpanded young leaves of each plant (Edwards et al. 1991). All DNA samples were treated with RNase. Out of 123 collected pairs of plants, sufficient quantities of DNA were extracted from both plants of 116 pairs and these 232 plants were included in the study.

### *Complementary survey 1997*

In June 1997, a complementary survey was carried out in the same area. Eligible farmers stating that they had plants of cultivars besides the ten most grown cultivars were included. Stem cuttings were collected from 45 plants identified by farmers as belonging to a cultivar that were not among the ten most grown studied in 1996. The stem-cuttings were transported to Sweden for molecular marker analysis as described above

## *Molecular marker analysis*

Seven short sequence repeat (SSR) primer pairs (Chavarriaga-Aguire et al. 1998) were used in multiplex polymerase chain reaction (PCR) amplifications. GA126, GA134, and GA136 were multiplexed using FAM labelled forward primers (set 1). GA57, GA127 and GA131 were multiplexed using HEX labelled forward primers (set 2), and GA161 using forward primers labelled with TET (set 3). Each reaction mix consisted of 2 µl of 10 ng/µl target DNA, 1 µl 10 x buffer, 0.4 µl 10 mM deoxynucleotide triphosphates (dNTPs), 2.5 pmol of each primer, 0.6 µl Dynazyme (Finnzyme) and H<sub>2</sub>O to 10 µl. Temperature cycling included denaturation at 95°C 3 min., followed by 35 cycles of denaturing at 93°C 1 min., annealing at 52°C 1 min. and extension at 72°C 1 min., and a final extension at 72°C 10 min.

After PCR amplification, pooling was done for each set of three PCR products in the following proportions: 1 µl set 1, 4 µl set 2, 0.6 µl set 3 and H<sub>2</sub>O to 20 µl. From the pool, 0.5 µl was mixed with 1.5 µl formamide, 0.5 µl GeneScan 500 (Perkin Elmer/Applied Biosystems) and 0.2 µl Blue dextran loading buffer. After denaturation, 1.5-2 µl was loaded on 6% denaturing gel (7 M urea) acrylamide: bisacrylamide (19:1) gels. The samples were separated by electrophoresis in 1 x TBE at 29 W for a minimum of 3 hours on an automatic DNA sequencer (Perkin Elmer/Applied Biosystems model 377XL). Allele sizes were determined with GeneScan version 2.1, and genotyping was performed manually from gel images. Each primer pair amplified one locus except GA161 that amplified two linked loci designated as GA161a and GA161b and the study thus included a total of 8 loci (Table 2).

## *Statistical analysis*

Fishers exact test and chi-square test was used to compare proportions. Principal component analysis was done on SSR allelic data. Each allele at each locus was treated as a separate variable (attaining values 0, 1 or 2). The least frequent allele (among all plants) at each locus was omitted in order to reduce dependencies between alleles at the same locus. A multivariate fit (JMP 1994, Multivariate fitting) was done on 7 of the 11 morphological variables of the 181 plants having any of the ten genotypes that was most frequent for each of the cultivars. Morphological variables that did not vary were excluded.

# RESULTS

Morphological description of the 18 to 26 plants identified as belonging to each of the 10 most grown cultivars are given in Table 1. The data on leaf shape and colour, inner root skin and colour of root pulp are not shown since no differences were observed among the 232 plants studied.

Most of the eight SSR loci included in the laboratory analysis were highly polymorphic (Table 2). More than one allele was common in each locus as shown by expected heterozygosities ranging from 0.30 to 0.74. The pattern of alleles found in these eight loci is regarded as the genotype for each plant studied.

The allele patterns of all genotypes found in the 232 plants are presented in Table 3. One single genotype was found in the majority (54-100%) of the 18 to 26 plants identified as belonging to each of the 10 most commonly grown cultivars. These genotypes are henceforth referred to as the "typical" genotype of that cultivar. In Table 3 the data from these genotypes are given in bold letters and the genotype code is given as "typical-" followed by a two-letter abbreviation for each cultivar name. Of the 181 plants with "typical" genotypes the farmers identified 14 as belonging to the wrong cultivar (Table 4). Table 3 also shows the allele composition of the other 29 genotypes found, henceforth referred to as "non-typical" followed by a two-digit number. Most of the plants with "non-typical" genotypes that were identified by farmers as belonging to the same cultivar had different "non-typical"

genotypes. Twelve “non-typical” genotypes were found in more than one plant, one in six, two in four, three in three and six in two. Four of these were found in plants identified as belonging to different cultivars of which two, 32 and 29, were found in plants referred to as belonging to both “cool” and “bitter” cultivars. A parent offspring analysis of the 51 plants with a “non-typical” genotype showed that for those classified as belonging to the cultivars ‘chimpuno’, ‘nyachikundi’ and ‘nyankhata’ more than half, 4/5, 3/5 and 7/8 respectively, could not be offspring. For the “non-typical” belonging to other cultivars the majority could be offspring, but since we only have recorded the genotypes for eight SSR markers the risk is high for type II errors, that is, an individual is accepted as progeny although it is not. On average the “non-typical” plants differed in five alleles from the “typical”, but three of the plants of the cultivar ‘ngwenyani’ had “non-typical” genotypes that only differed with one allele from the “typical”, suggesting that they may possibly have been cloned from a mutant plant.

The mean cyanogenic glucoside levels expressed as mg HCN equivalents per kg fresh weight were three to ten fold higher in plants with “bitter” “typical” genotypes compared to those of “cool” “typical” genotypes (Table 4). The cyanogenic glucoside levels in plants with “non-typical” genotypes, except a few, were several fold higher when identified as belonging to a bitter cultivar (Table 3).

Table 4 shows the percent agreement between the farmer’s identification of plants belonging to each name-given cultivar and the molecular marker identification of these plants having the “typical” genotype for that cultivar. The first column gives the farmers’ name of the cultivars and the second column the number of plants identified by farmers as belonging to that cultivar. Columns 3 - 12 show the number of plants with each “typical” genotype, named as a two-letter abbreviation of the corresponding cultivar. Columns 13 and 14 show the number of plants identified as belonging to each cultivar and having a “non-typical” genotype. The two bottom rows show the mean and standard error of cyanogenic glucoside levels of the roots of the plants for each genotype. The last column shows the percent agreement between the farmers’ identification of the plant as belonging to the particular cultivar and the molecular marker identification of the plants as having the “typical” genotype of that cultivar. The highest agreement between what the farmers said and the molecular marker findings were for ‘gomani’ (100%), ‘mbundumali’ (92%), and ‘nyamakozo’ (88%).

Farmers classified 170 plants as belonging to “bitter” and 62 as belonging to “cool” cultivars. The relative frequency of plants with a “non-typical” genotype was not significantly different between plants from the “bitter” or “cool” cultivars. The cyanogenic glucoside levels were much higher in plants classified as belonging to “bitter” cultivars compared to those classified as belonging to “cool” cultivars, irrespective of whether the genotype was “typical” or “non-typical”.

A comparison of farmers in Thowolo-B and Matyenda-1 shows that they differ in their cultivar maintenance. In an earlier interview study the farmers in Thowolo-B reported growing an average of 5.8 and those in Matyenda-1 an average of 3.6 cultivars (Chiwona-Karlton et al. 2000). In this study we found that the frequency of plants with “typical” genotypes is significantly lower in Thowolo-B (62%) than in Matyenda-1 (82%). However, this difference only apply for the cultivars ‘depweti’, ‘nyankhata’ and ‘nyamokzo’, for which 0/6, 7/18 and 5/8, respectively, were typical in Thowolo-B and 19/20, 8/8 and 16/16, respectively, were “typical” in Matyenda-1. One of the other cultivars, ‘ng’wenyani’, differed in the other direction, 13/20 being typical in Thowolo-B and 0/4 in Matyenda-1 and. The other six cultivars, which include all “cool” ones, did not differ between the two blocks. For three of the cultivars all the plants had a “typical” genotype and for four cultivars all but one plant have a “typical” genotype in “Matyenda-1”.

The allele pattern of the genotypes found in 45 plants collected in a complementary survey in 1997 is presented in Table 5. During this survey plants of the less common cultivars were collected. Farmers identified 20 plants as belonging to “cool” and 25 as belonging to “bitter” cultivars. Among these 45 plants 28 new genotypes were identified using DNA analysis and they are presented in Table 5 as consecutive numbers. Only three plants had a “typical” genotype of the ten most grown cultivars. Five plants had a “non-typical” genotype that was previously identified in the 1996 study among the ten

most grown cultivars studied in 1996. Although, some of the plants, collected from different farmers, had identical names, the genotypes were different except for two plants named 'kanonono'.

Figure 1 shows the results of the discriminate analysis of the seven varying morphological characters observed in the 181 plants with "typical" genotypes. The circles illustrate the morphological variation of each cultivar. There is a considerable morphological overlap between all cultivars except that of 'mbundumali'. This shows that the investigators were unable to separate the plants of the ten "typical" genotypes into different groups using the standard botanical descriptor.

The morphology of plants with the "typical" genotype of the "cool" cultivars 'chimpuno' and 'nyachikundi' overlaps with that of plants with "typical" genotypes of several "bitter" cultivars. The morphological overlap between the plants with "typical" genotypes of the seven "bitter" cultivars is particularly pronounced for the cultivar 'gomani' and 'nyamakozo' although Table 4 shows that farmers were able to separate all plants with these two genotypes.

Figure 2 shows a plot of the "typical" genotypes using the first two principle components of the composition of the SSR alleles of these ten genotypes. The genotypes of the three "cool" cultivars were genetically separated from those of the seven "bitter" cultivars. Already the first principal component was significantly ( $p < 0.001$ ) different for the two groups. All 39 "typical" and "non-typical" genotypes found in plants identified as belonging to "cool" and "bitter" cultivars were also clustered into two significantly ( $p < 0.001$ ) different groups in the principle component analysis.

## DISCUSSION

Cassava (*Manihot esculenta* Crantz) is an important staple crop in Africa (Cock 1982; Romanoff 1992). Wherever cassava is of significant importance as a staple crop the "bitter" cultivars with high cyanogenic glucoside content dominate (Jones 1959; Purseglove 1968; Fresco 1986). These compounds may cause adverse effects if not reduced to negligible levels during processing (Tylleskär et al. 1992). Advances in molecular genetics now offer the possibility to develop acyanogenic cassava (IITA 1993; Dixon et al. 1994; Keresztessy et al. 1994a; Keresztessy et al. 1994b; McMahon et al. 1995; Andersen et al. 2000) but its relevance to small-scale farmers remains controversial (Wambugu 1999; Machuka 2001). To partly clarify this issue we used molecular genetics to elucidate how cassava farmers understand and manage cassava genetic diversity in relation to cassava cyanogenesis. Malawian women farmers were studied since they, like many other small-scale farmers in Africa, grow a repertoire of both "cool" and "bitter" cassava cultivars in the same field (Chiwona-Karltun et al. 1998) that morphologically appear to be similar. Roots from "cool" cultivars are regarded as non-toxic, whereas those of "bitter" cultivars are regarded as toxic and requiring extensive processing that, as a norm, is done solely by women (Chiwona-Karltun et al. 1998; Chiwona-Karltun et al. 2000). Despite this additional labour and time requirement for processing these farmers still prefer 'bitter' cultivars since toxicity is perceived as conferring protection from theft. Furthermore, these farmers claim that they are able to distinguish plants from the "bitter" and the "cool" cultivars by looking at their morphological appearance. The present study combined participatory farmer (Sperling & Sheidegger 1995; Fujisaka 1999) guided plant collection with laboratory DNA assay using PCR amplified SSR markers (Chavarriaga-Aguire et al. 1998). The quality of comparisons between laboratory DNA assays and the farmers' ability to identify the genetic diversity of their crop depend equally on the methodological stringency in the field survey and in the laboratory analysis. Mutual rapport was a fundamental issue when requesting information from the farmers. This study entailed tedious activities for the farmer and the investigators therefore remunerated them for the pre-emptory harvest of their cassava. The SSR, or microsatellite, DNA sequences mutate at rates several orders of magnitude higher than that of the bulk of DNA. SSR-markers are therefore very informative for assessment of intra-species genetic diversity (Chavarriaga-Aguire et al. 1999). In the main study of 232 plants of the ten most grown cultivars we found it sufficient to use eight SSR loci since each one had high poly-

morphism. A complementary survey of the less common cultivars in 1997 was conducted to confirm that the eight SSR loci were sufficient to separate the genotypes grown in the area. Scoring with the same eight SSR loci in DNA from the 45 plants collected identified 27 new genotypes. Only three plants had the “typical” genotype of the ten most grown cultivars, which is what one could expect being caused by farmers’ misidentification.

### *Cultivar and genotype*

The finding of one single genotype in 92 to 100% of the plants that farmers identified as belonging to the two most grown cultivars, ‘mbundumali’ and ‘gomani’, strongly indicate that the studied farming communities endeavour to maintain the most common cultivars as single clones. It would appear that farmers strive to maintain each of the ten most grown cultivars as one specific genotype as 72% of all plants, 167 of 232, had the typical genotype of the cultivar it was identified to belong to. Among plants not having the “typical” genotype of each cultivar only 6%, 14 of 232 had a “typical” genotype of another cultivar. More common was that the lack of agreement of the results between farmers and molecular marker identification was due to a “non-typical” genotype. This was found in 22% of the plants, 51 of 232. Although the farmers in these two blocks appear to strive for cultivars consisting of single clones their fields contain a considerable proportion of plants of more than 30 “non-typical” genotypes that farmers fail to distinguish from the “typical” genotypes of the ten most common cultivars. Since farmers were free to identify plants that they considered as belonging to any of the ten named cultivars the results do not tell the proportion of plants with “non-typical” genotypes in their fields. However, among the plants considered to belong to one of the ten most common cultivars the “typical” genotype was always at least three times more frequent than were any of the “non-typical” genotypes.

All “non-typical” genotypes were found in low frequencies and only four of the non-typical genotypes (07, 11, 14 & 15) were found in plants identified as belonging to different cultivars (Table 3). Mutations in the SSR loci may only explain a few of the “non-typical” genotypes in the main survey since merely 8 of the 51 plants with “non-typical” genotypes differed with only a single allele when compared with the “typical” genotype. We cannot exclude that some “non-typical” genotypes are offsprings to the “typical” genotypes. We deduce that farmers manage to maintain the most preferred cultivars as single genotypes but that high proportions of plants of the less grown cultivars have “non-typical” genotypes. Our findings have similarities with recent findings from a study of American Indian cassava farmers in Guyana (Elias et al. 2000; Elias et al. 2001).

The difference in the number of plants with “typical” genotypes between the two studied communities, situated only 3 km apart, suggests that a high agreement between named common cultivars and one specific genotype may be a local phenomenon. The cultivar ‘depwete’ was reported as being recently adopted in “Thowolo B” (Chiwona-Karlton et al. 2000), and none of the reported six plants identified as ‘depweti’ had the “typical” genotype. Whereas in Matyenda-1 where ‘depwete’ was frequently grown since many years back all but one of 20 identified plants had the “typical” genotype. It is noteworthy that the “Matyenda-1” farmers that reported growing fewer cultivars managed to identify a higher proportion of plants (82%) with “typical” genotypes. In addition to ‘mbundumali’ and ‘gomani’ that had high agreement in both communities the cultivars ‘depwete’, ‘nyankhata’ and ‘nyamakozo’ had similarly high agreement in Matyenda-1. This further supports that small-scale cassava farming communities strive to maintain distinct cultivars that are single genotypes. An equally high agreement between the farmers’ cultivar concept and one single genotype may well be found in most of the small-scale cassava farming systems in Africa. However, the probability that specific agreement between cultivar name and genotype may be restricted to quite small areas cannot be excluded. The names of the most popular cultivars, ‘gomani’ and ‘mbundumali’, are referred to over large parts of Malawi. It remains to be shown if the use of these names refers to the same genotypes in other parts of Malawi.

Molecular markers can help breeding and extension programmes to understand the contemporary pat-

tern of genotypes used in the small-scale farming communities. This would require collection of plant material in a participatory interaction with local farmers and analysis of DNA from several plants of each common cultivar from each community before drawing generalisable conclusions between name and genotype.

### *Farmers accuracy in identifying cultivars and genotypes*

Our morphologic classification of plants was based on a modified botanical key for cassava (Nweke et al. 1994). Notwithstanding the discriminate analysis using the seven differentiating morphological characters for all plants with “typical” genotype showed that the plants with the “typical” ‘mbundumali’ genotype were the only group that could be distinctly differentiated (Figure 1). However, when the farmers examined the plants they could with relative ease differentiate 167 (92 %) of the 181 plants with “typical” genotype. If the ‘depwete’ genotype the farmers’ only major problem, is excluded they correctly differentiated 97% of the remaining 153 plants with nine different “typical” genotypes. The farmer’s skilled accuracy was further supported by the findings in the complementary study in 1997. Only 3 out of 45 plants identified as belonging to less common cultivars turned out to have a “typical” genotype of one of the ten most grown cultivars. Once more if ‘depweti’ is excluded, the farmers’ mistakes occurred for less than 3% of the plants. It seems most peculiar that farmers could correctly identify 43 (93%) plants of the 46 with “typical” genotypes of ‘gomani’ and ‘nyamakozo’, since when using the botanical key we found considerable morphological overlap between the plants of these two genotypes (Table 1 & Figure 1).

The reason for the farmers high accuracy in identifying ‘gomani’ and ‘nyamakozo’ plants cannot be attributed to the plants of these two genotypes being very common in the fields, since ‘nyamakozo’, was the least grown cultivar of the ten (Chiwona-Karlton et al. 2000). Neither can the high accuracy be attributed to the farmers having planted cultivars in some easily recognisable pattern in the fields. We clearly observed that the farmers’ identification of the plants of each cultivar always involved thorough searching throughout each field and a careful examination of a considerable number of plants. The farmers’ high accuracy in identifying plants with the “typical” genotypes of the ten most common cultivars is most probably explained by their use of more detailed morphological characters than the ones used by the investigators in this study. The local classification of cassava plants into cultivars with local names thus reflects a skilful morphological recognition of several locally preferred genotypes (Chiwona-Karlton et al. 2000). Farmers in this part of Africa have cultivated cassava for only one to two centuries (Jones 1959; Carter et al. 1992). In spite of this they appear to be as good at identifying their local cassava genotypes as the Amerindians that have been growing cassava for several thousands of years (Boster 1985; Bellon 1996; Salick et al. 1997; Moore 1998). Careful use of botanical keys developed for the whole species cannot match the accuracy of local farmers in morphological differentiation of their most common cassava cultivars.

These findings would imply that a cultivar would not be adopted within a farming system if it cannot be morphologically identifiable from the cultivars already grown in the area (Boster 1985). A cassava cultivar thus needs to be recognised as a distinct morphological type before its yield, taste and other qualities can be assessed in the local environment. This has also been reported from cassava farming systems in South America (Boster, 1985; Sambatti et al. 2001). Studies in the Amazonian basin of Peru have shown that farmers select cultivars using perceptual taxonomic characters that show the greatest variation i. e. leaf shape, petiole colour and stem colour (Boster 1985; Salick et al. 1997; Elias et al. 2000; Sambatti et al. 2001).

In addition to the handful of cultivars occupying most of the land planted with cassava the small-scale cassava farming system also preserve a high genetic bio-diversity of cassava. This is done by maintaining some plants of many old name-given cultivars and by testing new name-given cultivars from outside their community. It is also done by collecting new *volunteer* seedlings from sexually propagated cassava in their fallow fields (Chiwona-Karlton et al. 1998; Chiwona-Karlton et al. 2000). The DNA analysis suggests the least grown cultivars kept for bio-diversity are more genetically hete-

rogeneous than the common cultivars. This is in accordance with what has been found in small-scale Indian potato farming systems (Johns & Keen 1986) and in cassava diversity studies (Bellon 1996; Salick et al. 1997). Studies in small-scale Indian cassava farming systems in South America using morphological classification of plants suggest that farmers in the same community may differ in their accuracy in recognising cultivars (Boster 1984; Boster 1985; Salick et al. 1997; Sambatti et al. 2001). Beside the differences observed between the two communities we could not find any such pattern among the 28 farmers included in this study,

### *Differences between “bitter” and “cool” cultivars*

Farmers classified 8% of the plants with “typical” genotype as belonging to the wrong cultivar, but all 14 mistakes were made among the seven “bitter” cultivars. We saw no cases whereby the farmers misclassify plants with “typical” genotypes as “cool” when they were supposed to be “bitter” or vice-versa. The root cyanogenic glucoside levels in the “typical” plants of “bitter” cultivars was four to seven fold higher (Table 3 and 4) compared to the “cool” cultivars with “typical” genotypes. Only two of 11 “non-typical” genotypes found among plants identified as belonging to “cool” cultivars were also found among plants identified as belonging to “bitter” cultivars (Table 3). The mean glucoside levels in roots of the plants with “non-typical” genotype were three fold higher for those classified as belonging to “bitter” cultivars than those classified as “cool” cultivars. These findings indicate that farmer’s ethno-classification of cassava cultivars into “cool” and “bitter” reflects their knowledge of the potential risk for poisonous effect from cyanogenic glucosides in the roots.

These differences between “cool” and “bitter” cultivars supports the results from interview studies in which the farmers in this part of Malawi were found to regard “bitter” and “cool” cassava cultivars as two different crops in their farming and food system (Chiwona-Karltun et al. 1998; Chiwona-Karltun et al. 2000). They maintained that roots from the “cool” cultivars were safe for consumption in the raw form whereas roots from the “bitter” group of cultivars could only be consumed safely as the staple food *kondowole* after processing by soaking and fermentation, drying and pounding into flour (Chiwona-Karltun et al. 2000). This method of processing (Lancaster et al. 1982; Dufour 1989) reduces cyanogenic compounds to negligible levels (Banea et al. 1992).

The genotypes of the “cool” cultivars were separated from those of the “bitter” cultivars (Figure 2). This suggests that the farmers’ necessity to differentiate between “cool” and “bitter” cultivars might have influenced the genetic structure of cassava by the way farmer over time decides which cultivars to plant and which ones to discard (Wilson & Dufour *In Press*). The division observed in contemporary Malawi might have occurred locally, elsewhere in Africa, or already in South America many centuries to millenniums ago. A more speculative hypothesis is that the two groups of cultivars constitute separate domestication (Allem 1994; Dufour, 1995; Allem 1999; Rogers & Fleming 1973; Ugent et al. 1986). It should be noted that Amerindians also appear to regard “bitter” and “sweet” cultivars as two different crops (Box & Box-Lasocki 1982; Sauer 1963; Dufour 1993; Dufour 1995). However, studies using botanical taxonomy have not recognised any morphological signs of genetic division (Nordenskiöld 1924; Rogers 1965; Renvoize 1972; McKey & Beckerman 1993). This supports Boster’s hypothesis (Boster 1985) that morphologic characters do not relate to agronomic characters since differences in morphology are an effect of farmers’ need to perceptually distinguish cultivars.

## CONCLUSION

We conclude that the biological knowledge of cassava cyanogenesis among small-scale African farmers enables them to benefit from its protection against theft without any risk as the toxicity can be removed by processing that takes almost one week. The potential thieves, predominantly hungry young males, are deterred by the duration of processing that is traditionally done only by women. Dietary cyanide exposure from cassava is rare, and causes severe health effects when short cuts in processing brought about by food shortages induced by environmental degradation, drought, severe poverty or war. Ironically, toxic effects are most common where protection against theft is most needed. As the farmers at risk will not plant non-toxic cultivars, transgenic acyanogenic cassava will not help much in prevention of cassava toxicity. Notwithstanding, acyanogenic cassava may be useful where theft is rare and “cool” roots are consumed without processing, as well as to further the understanding of the biology of cyanogenesis. Further analyses of the genetic differences between “cool” or “sweet” and “bitter” cultivars will tell how and when they became genetically separated. The differentiation may have affect many agronomic characters and is therefore important to consider in the maintenance of breeding populations. It seems to be taxonomically important for cassava farmers that the new cultivars fit into either of these two categories. Scientists need to understand the mechanisms that determine the adoption of new cultivars.

Biotechnology indisputably has the potential to improve food security among small-scale farmers in Africa by the use of tissue culture, marker assisted breeding and development of transgenics. We advocate that the advances in molecular genetics also should be used to improve the understanding of farming and food systems of poor small-scale farmers by combined use of in-depth interviews, molecular techniques and farmer participation.

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Table 1. Distribution of morphological categories within each character of the plants classified by farmers as belonging to the ten most grown cultivars.

Cultivar	No. of plants	Morphological characters with 2-3 categories <sup>a</sup>														
		Skin colour		Stem colour		Shoot colour		Shoot pubesc		Lamina colour		Petiole colour		Root neck constriction		
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	3
Mbundumali	24	24	0	24	0	17	4	<u>0</u> <sup>b</sup>	<u>21</u>	23	0	<u>20</u>	<u>3</u>	8	12	4
Non-typical <sup>c</sup>		2	0	2	0	1	1	0	2	2	0	1	1	1	1	0
Chimpuno	18	9	9	17	1	4	14	2	16	<u>6</u>	<u>11</u>	4	14	10	7	1
Non-typical	5	1	4	4	1	0	5	2	3	3	2	3	2	2	2	0
NyaChikundi	20	0	20	<u>10</u>	<u>8</u>	<u>7</u>	<u>11</u>	<u>12</u>	<u>6</u>	<u>12</u>	<u>6</u>	0	20	5	13	2
Non-typical	5	0	5	3	2	<u>2</u>	<u>2</u>	<u>1</u>	<u>3</u>	5	0	0	5	1	4	0
Gomani	22	0	22	8	14	1	21	18	4	<u>16</u>	<u>5</u>	<u>9</u>	<u>11</u>	8	13	1
Depweti	26	<u>21</u>	<u>4</u>	<u>25</u>	<u>0</u>	<u>4</u>	<u>19</u>	<u>21</u>	<u>2</u>	24	2	<u>4</u>	<u>20</u>	13	9	2
Non-typical	6	<u>2</u>	<u>3</u>	6	0	1	5	5	1	6	0	2	4	2	4	0
typ -Ng	1	0	1	<u>0</u>	<u>0</u>	1	0	1	0	1	0	1	0	0	1	0
Koloweki	24	23	1	24	0	<u>1</u>	<u>22</u>	<u>15</u>	<u>8</u>	<u>20</u>	<u>2</u>	<u>14</u>	<u>8</u>	7	10	7
Non-typical	5	5	0	5	0	<u>0</u>	<u>4</u>	<u>3</u>	<u>1</u>	5	0	4	1	0	5	0
typ - De	5	4	1	5	0	1	4	4	1	5	0	2	3	5	0	0
NyaHarawa	24	2	22	11	13	4	20	10	14	22	2	9	15	13	9	2
Non typical	8	0	8	1	7	1	7	2	6	8	0	5	3	6	1	1
typ -De	1	1	0	1	0	0	1	1	0	1	0	0	1	1	0	0
typ - Go	1	0	1	1	0	0	1	0	1	1	0	0	1	0	0	1
typ - Nk	1	1	0	1	0	0	1	1	0	1	0	0	1	1	0	0
NyaNkhata	26	19	7	21	5	0	26	18	8	18	8	<u>9</u>	<u>16</u>	8	18	0
Non typical	8	7	1	8	0	0	8	4	4	8	0	4	4	4	4	0
typ - De	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
typ - Go	1	0	1	1	0	0	1	0	1	0	1	0	1	0	1	0
typ - Nm	1	1	0	1	0	0	1	1	0	1	0	0	1	0	1	0
Ng'wenyani	24	7	18	22	2	<u>0</u>	<u>23</u>	<u>14</u>	<u>9</u>	<u>19</u>	<u>4</u>	13	11	9	10	5
Non typical	9	3	6	7	2	<u>0</u>	<u>8</u>	<u>3</u>	<u>5</u>	7	2	5	6	4	2	3
typ - De	2	2	0	2	0	0	2	2	0	1	1	1	1	2	0	0
Nyamakozo	24	0	24	<u>4</u>	<u>18</u>	<u>0</u>	<u>23</u>	<u>23</u>	<u>0</u>	23	1	<u>16</u>	<u>7</u>	3	12	9
Non-typical	3	0	3	1	2	0	3	3	0	3	0	2	1	0	3	0

- a. **skin colour** = outer root skin colour (1 = white/cream, 2 = brown); **stem colour** = mature stem colour (1 = white/grey, 2 = brown); **shoot colour** (1 = green, 2 = green-purple or purple); **Shoot pubesc** = shoot pubescence (1 = absent, 2 = present); **Lamina colour** = leaf lamina colour (1 = green, 2 = green-purple or purple); **petiole colour** (1 = green, 2 = green-purple or purple); **root neck constriction** (1 = short, 2 = intermediate, 3 = long).
- b. Underlined number indicates that data is missing for this variable for 1 to 3 plants.
- c. Numbers in italic are for those of the plants identified as belonging to each cultivar found to have a typical genotype of another cultivar or a non-typical genotype.

**Table 2. Allele frequencies in genotypes found in plants identified as belonging to three ‘cool’ and seven ‘bitter’ cultivars.**

SSR locus <sup>a</sup>	Allele (bp <sup>b</sup> )	Cultivar type		P <sup>c</sup>
		cool	bitter	
GA161a	105	0.89	0.80	0.524
GA161b	131	0.39	0.72	0.006
	129	0.35	0.08	0.008
GA131	118	0.27	0.46	0.140
	116	0.35	0.01	0.013
	114	0.27	0.14	0.216
	106	0.12	0.18	0.530
GA57	183	0.23	0.20	0.773
	179	0.46	0.52	0.639
	160	0.23	0.28	0.786
GA127	232	0.62	0.86	0.021
	230	0.12	0.04	0.331
	228	0.08	0.00	0.120
	216	0.12	0.08	0.685
GA136	151	0.69	0.66	0.804
GA126	219	0.15	0.08	0.434
	213	0.19	0.12	0.496
	183	0.12	0.10	0.999
	181	0.46	0.66	0.140
GA134	319	0.85	0.76	0.555
	309	0.12	0.24	0.238

- a. From each locus the least frequent allele was omitted (i.e., GA161a - 99; GA161b - 123; GA131 - 96; GA57 - 181; GA127 - 240; GA136 - 187; GA134 - 333).
- b. bp is number of base pairs in each allele.
- c. The probability (P) of allele frequency homogeneity by Fisher’s Exact Test.

Table 3. Allele composition of genotypes in 232 plants of ten cultivars collected in 1996.

Cultivars	Genotypes	No. of plants	ALLELES AT THE EIGHT SSR GA-LOCI								HCN <sup>a</sup> mean
			161a	161b	131	57	127	136	126	134	
Mbundumali	typical-Mb	22	11	23	23	13	13	12	14	22	26
	non-typical-01	1	11	12	24	23	13	12	14	22	22
	non-typical -02	1	11	23	22	23	12	11	24	22	15
Chimphuno	typical-Ch	13	11	23	12	23	12	12	24	22	25
	non-typical -03	1	11	12	34	23	11	12	14	22	39
	non-typical -04	1	11	22	12	14	11	12	44	23	26
	non-typical -05	1	11	23	12	14	11	12	24	22	32
	non-typical -06	1	11	23	13	23	23	12	24	22	93
	non-typical -07	1	12	11	14	12	11	11	13	12	66
NyaChikundi	typical-Nc	15	11	13	23	22	14	11	24	22	32
	non-typical -08	2	11	13	23	22	14	11	44	22	17
	non-typical -09	1	12	11	13	12	15	11	35	12	39
	non-typical -10	1	12	11	13	12	15	12	35	12	79
	non-typical -11	1	12	11	45	23	11	11	34	12	38
Gomani	typical-Go	24 <sup>b</sup>	12	11	15	12	11	11	34	11	163
Depweti	typical-De	28 <sup>b</sup>	12	11	14	12	11	11	44	12	111
	non-typical -12	4	11	11	14	13	11	12	34	12	132
	non-typical -13	1	11	12	14	23	13	12	14	22	83
	non-typical -14	1	11	23	12	23	11	12	14	22	113
Koloweki	typical-Ko	14	11	12	24	23	11	11	14	22	123
	non-typical -15	1	11	13	15	13	11	11	23	12	193
	non-typical -16	1	11	13	23	23	11	11	34	22	95
	non-typical -17	2	11	13	34	23	12	12	24	22	66
	non-typical -18	1	12	33	15	23	11	12	45	12	107
NyaHarawa	typical-Nh	13	11	11	13	12	11	22	44	22	112
	non-typical -19	4	11	11	11	22	15	12	44	22	79
	non-typical -20	3	11	11	13	12	11	12	44	22	151
	non-typical -21	1	12	33	13	12	11	22	44	22	102
NyaNkhata	typical-Nk	16 <sup>b</sup>	12	33	15	13	11	12	45	12	203
	non-typical -07	2	12	11	14	12	11	11	13	12	43
	non-typical -15	4	11	13	15	13	11	11	23	12	112
	non-typical -22	1	11	23	12	23	11	12	14	22	88
	non-typical -23	1	12	11	12	23	11	12	14	22	79
Ng'wenyani	typical-Ng	14 <sup>b</sup>	11	11	11	22	13	12	24	22	177
	non-typical -14	1	11	23	12	23	11	12	14	22	165
	non-typical -15	1	11	13	15	13	11	11	23	12	167
	non-typical -24	2	11	11	11	22	13	12	24	12	148
	non-typical -25	1	11	11	11	22	13	12	44	22	220
	non-typical -26	1	11	12	33	23	12	12	22	22	101
	non-typical -27	2	12	11	12	12	11	11	44	12	166
	non-typical -28	1	12	11	14	12	11	11	44	22	74
Nyamakozo	typical-Nm	22 <sup>b</sup>	11	11	14	23	11	11	44	12	251
	non-typical -11	2	12	11	45	23	11	11	34	12	151
	non-typical -29	1	12	11	45	23	11	11	44	12	127

a. Mean levels of cyanogenic glucosides are given as mg HCN equiv. per kg dry weight

b. Include plants classified to other cultivars (see Table 4).

**Table 4. Agreement between farmer’s identification of cultivar and laboratory identification of genotypes of 232 plants.**

Cultivars <sup>a,b</sup>	No. of plants	Number of plants with genotypes that are:											Agree- ment <sup>c</sup>	
		typical										non-typical		
		Mb	Ch	Nc	Go	De	Ko	Nh	Nk	Ng	Nm	cool		bitter
<b>Cool</b>														
Mbundumali(Mb)	24	22										2		92
Chimpuno (Ch)	18		13									5		72
NyaChikundi(Nc)	20			15								5		75
<b>Bitter</b>														
Gomani (Go)	22				22								0	100
Depweti (De)	26					19				1			6	73
Koloweki (Ko)	24					5	14						5	58
NyaHarawa (Nh)	24				1	1		13	1				8	54
NyaNkhata (Nk)	26				1	1			15		1		8	58
Ng'wenyani (Ng)	24					2				13			9	54
Nyamakozo (Nm)	24										21		3	88
<b>Total</b>	232	22	13	15	24	28	14	13	16	14	22	12	39	
HCN mean <sup>d</sup>		26	25	32	163	111	123	112	203	177	251	40	119	
HCN s.e. <sup>d</sup>		4	7	7	17	7	11	16	19	28	26	26	4	

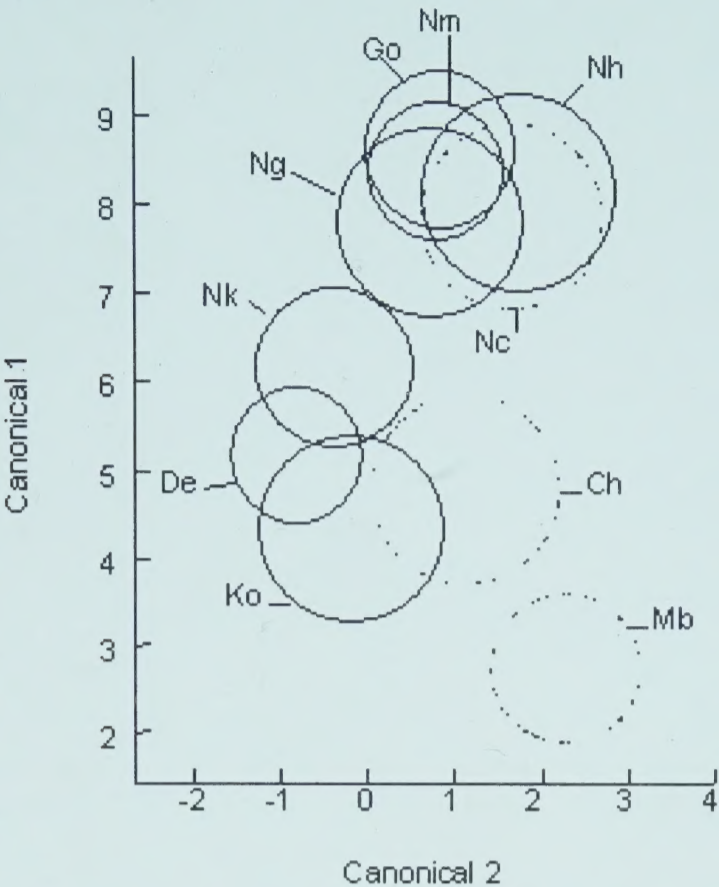
- a. Within ‘cool’ and ‘bitter’ groups, cultivars are listed in descending order according to the proportion of farmers in the area growing the cultivar
- b. The abbreviation in parenthesis designates the “typical” genotype of each cultivar.
- c. Percentage of plants with “typical” genotype among plants identified as belonging to each cultivar.
- d. Mean and s.e. of the level of cyanogenic glucosides in root parenchyma of all plants belonging to each “typical” genotype and plants with “non-typical” genotypes among “cool” and “bitter” cultivars, respectively, expressed as HCN equivalents per kg fresh weight.

Table 5. Alleles of genotypes of 45 plants collected in complementary survey in 1997

Cultivar		Genotype <sup>a</sup>	Alleles at the eight SSR GA-loci							
Name	Taste		161a	161b	131	57	127	136	126	134
Chibisa	Cool	1	12	23	13	23	12	22	25	22
Fyoka	Cool	2	11	33	34	23	25	11	25	22
Fyoka	Cool	3	12	33	11	12	12	12	23	12
Kabinyu	Cool	4	11	33	22	22	13	12	44	22
Kachamba Mtuba	Cool	5	11	13	34	12	11	12	11	22
Kalomu	Cool	6	11	22	14	22	13	12	45	22
Kamwala	Cool	4	11	33	22	22	13	12	44	22
Kanjuchi	Cool	7	11	22	23	12	11	12	45	22
Kanonono	Cool	8	12	33	11	12	12	12	35	22
Kanonono (New)	Cool	9	11	12	13	23	13	11	25	22
Kweti Chimulole	Cool	10	12	22	13	12	11	12	35	12
Mnyakayuni	Cool	11	11	23	13	23	12	22	25	22
Mnyakayuni	Cool	4	11	33	22	22	13	12	44	22
Mwaya	Cool	typ-Ch	11	23	12	23	12	12	24	22
Ng'ung'uta	Cool	4	11	33	22	22	13	12	44	22
Ntheghama	Cool	12	11	12	33	23	11	11	14	22
Nyasungwi	Cool	13	12	11	14	12	11	11	44	11
Palamu	Cool	14	12	11	14	12	11	11	13	12
Palamu	Cool	typ-De	12	11	14	12	11	11	44	12
Virginia	Cool	12	11	12	33	23	11	11	14	22
20:20	Bitter	15	11	11	34	22	11	12	14	22
Cakubaba	Bitter	non-typical-03	11	12	34	23	11	12	14	22
Chigwalantha	Bitter	16	11	22	36	33	12	11	25	12
Ching'anya	Bitter	17	11	23	22	23	25	12	15	22
Gomani Admarc	Bitter	non-typical-15	11	13	15	13	11	11	23	12
Kachamba Muyera	Bitter	18	12	22	13	12	15	12	35	12
Kachamba Muyera	Bitter	19	11	13	15	13	12	12	23	12
Kachamba Muyera	Bitter	20	11	12	35	12	11	12	45	12
Kalomu	Bitter	typ-De	12	11	14	12	11	11	44	12
Kanonono (old)	Bitter	9	11	12	13	23	13	11	25	22
Kanonono (old)	Bitter	11	11	23	13	23	12	22	25	22
Kawalika	Bitter	15	11	11	34	22	11	12	14	22
Kolobeki Chibala	Bitter	21	11	12	12	13	13	12	14	22
Kweti Chimulole	Bitter	22	11	11	11	23	13	12	45	22
Mbayani	Bitter	non-typical-15	11	13	15	13	11	11	23	12
Mnyakayuni	Bitter	23	11	11	11	13	11	22	45	22
Mpuma	Bitter	22	11	11	11	23	13	12	45	22
Mpuma	Bitter	24	11	13	15	11	12	12	23	12
Mwatatu	Bitter	25	11	34	33	13	13	12	13	22
Nyankhonjerwa	Bitter	26	11	11	13	12	12	11	23	12
Nyasalima	Bitter	27	11	23	33	13	12	22	24	22
Palamu	Bitter	non-typical-15	11	13	15	13	11	11	23	12
Thipula	Bitter	7	11	22	23	12	11	12	45	22
Thipula	Bitter	28	11	12	13	23	11	12	15	22

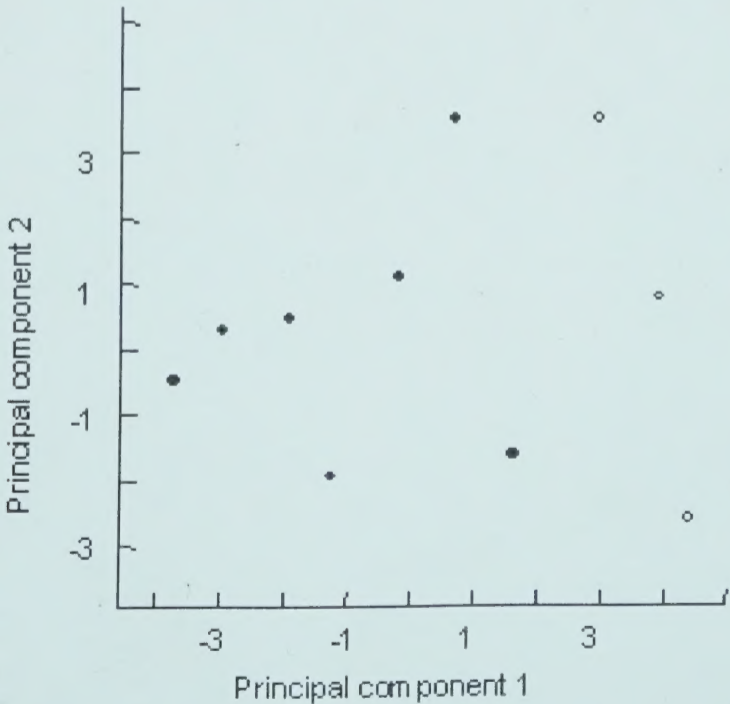
Genotype in bold occurs in more than one plant. Numbers from 1 to 27 indicates genotypes found in 1997. The others are as labelled in Table 3.

Figure 1. Centroid plots of the first two canonical variables from discriminate analysis. The analysis was done on seven morphological variables of the 13 to 28 plants with typical genotypes of the three 'cool' (dashed perimeter) and seven 'bitter' (continuous perimeter) cultivars. The abbreviations (as in Tables 3&4) indicate each genotype and the perimeter marks the 95% confidence region.



**Figure 1.** Discriminate analysis of seven varying morphological characters observed in the 181 plants with "typical" genotypes.

Figure 2. Scattergram based on principle component analysis of the typical genotypes of the three 'cool' (°) and seven 'bitter' (l) cultivars. The analysis is done on the allelic composition of eight SSR loci. The first component explains 36% and the second 18% of the total variation. The abbreviations for the genotypes are those used in Table 2.



**Figure 2.** Plot of the "typical" genotypes using the first two principle components of the composition of the SSR alleles of these ten genotypes.





